

Structural Biology with X-Rays:

*Combining Biology, Chemistry, Physics,
and Mathematics to do Science*

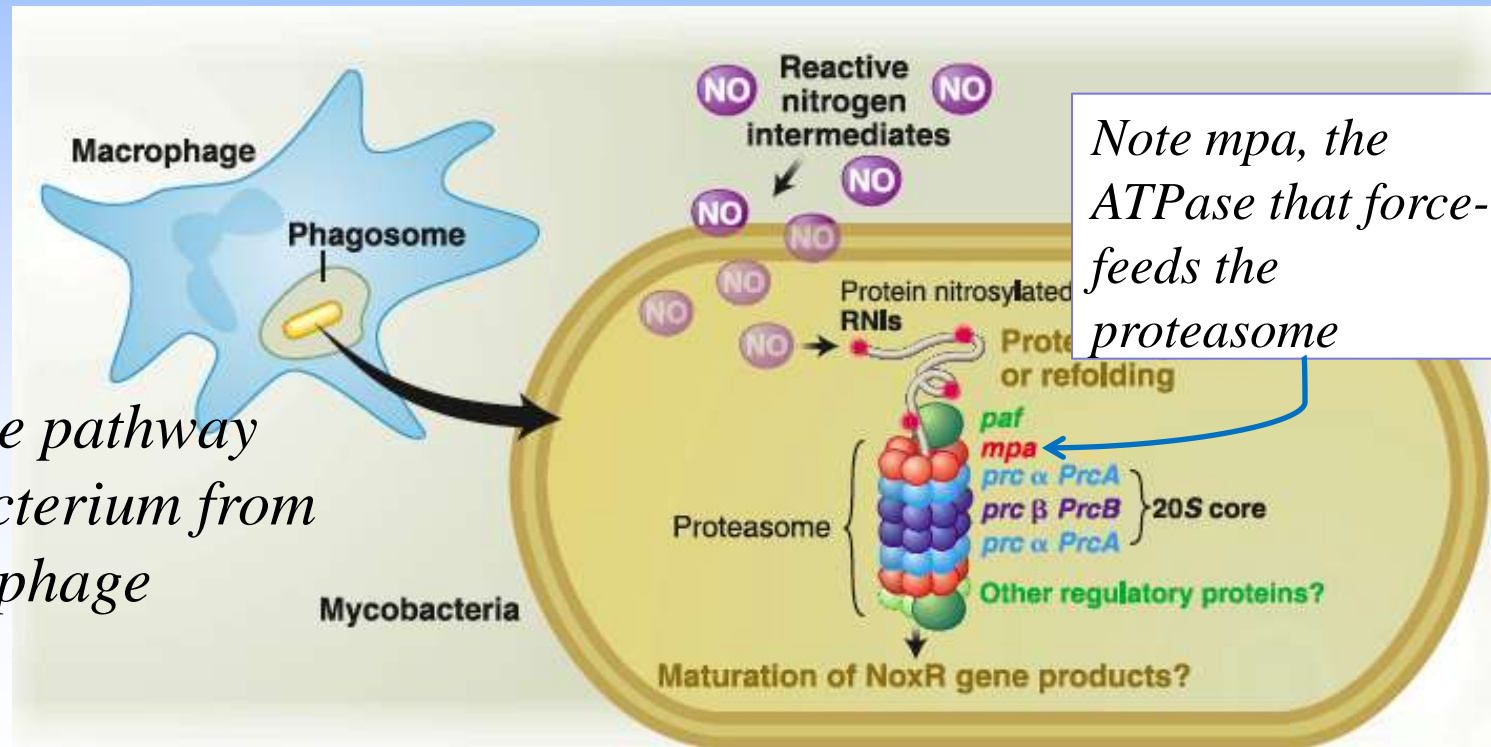
R.M. Sweet
Biology Department
Brookhaven National Laboratory

The Plan for this Lecture

I'm going to take you through some of the adventures and successes scientists in the **Brookhaven Biology Department** have had in using some of Brookhaven's unique facilities to make useful discoveries.

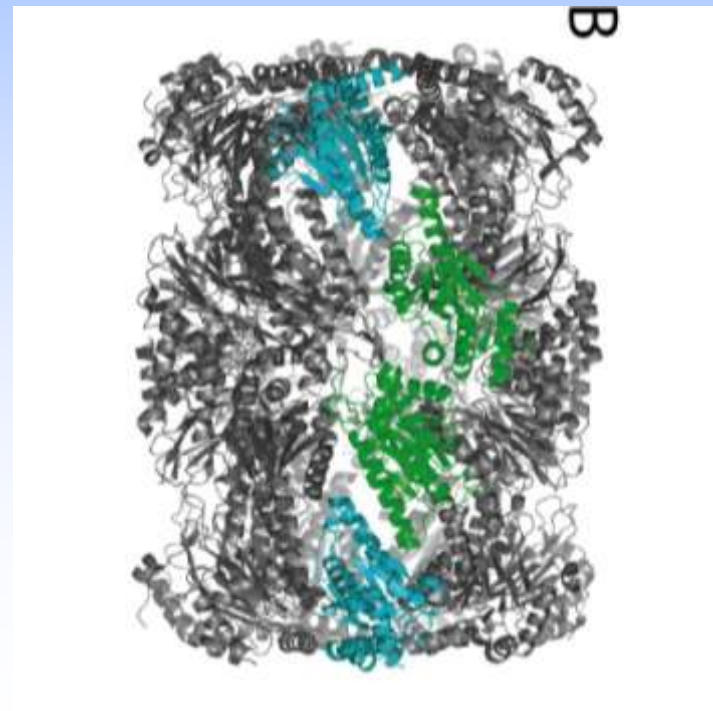
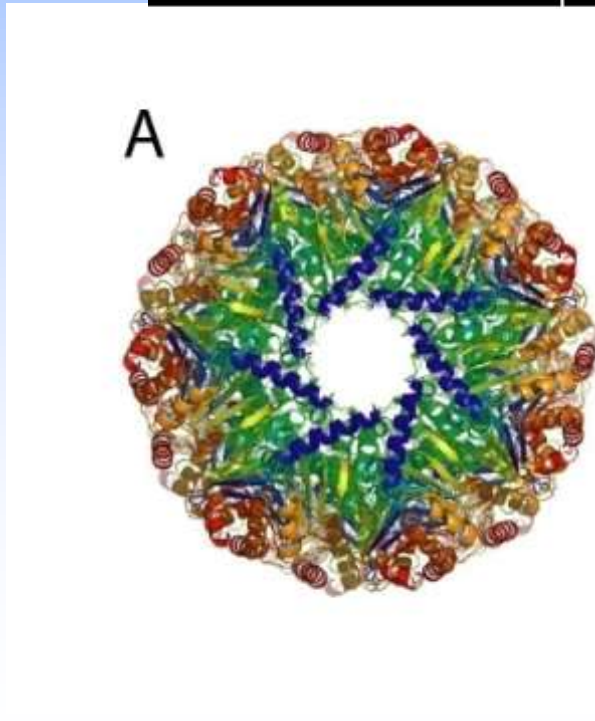
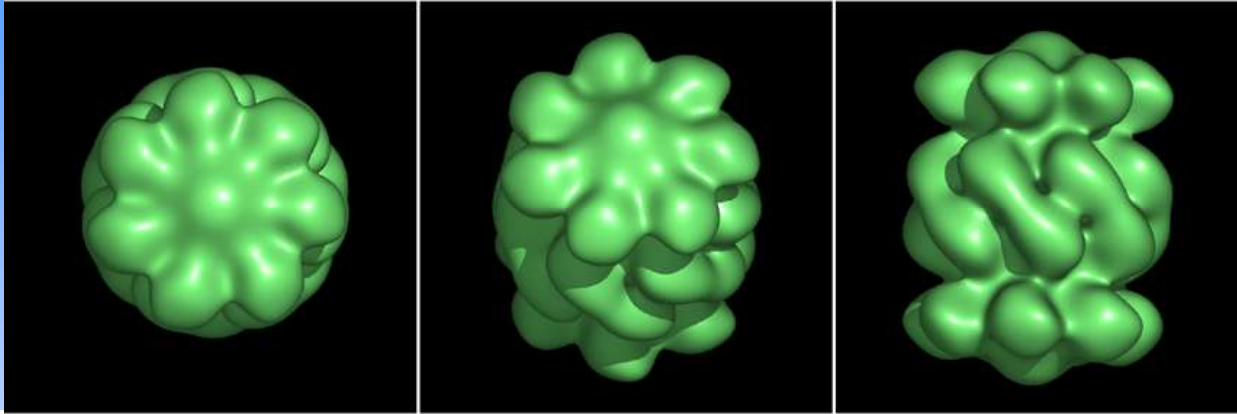
For example....

*The group of Hui-Lin Li of BNL
Biology study a defense
mechanism of Mycobacterium
tuberculosis. To defeat it could
provide a cure for Tb*

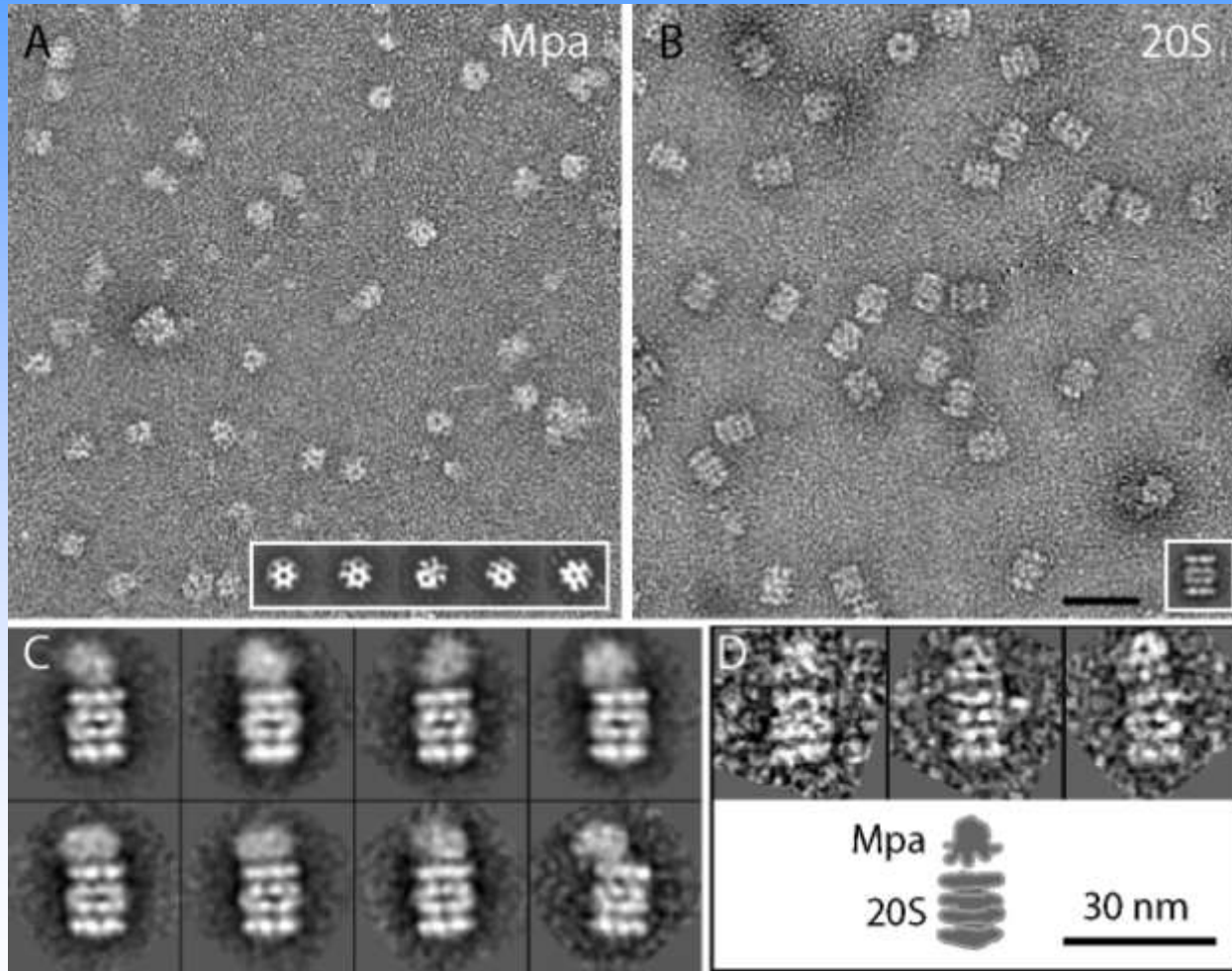


*The proteasome pathway
defends the bacterium from
the host macrophage*

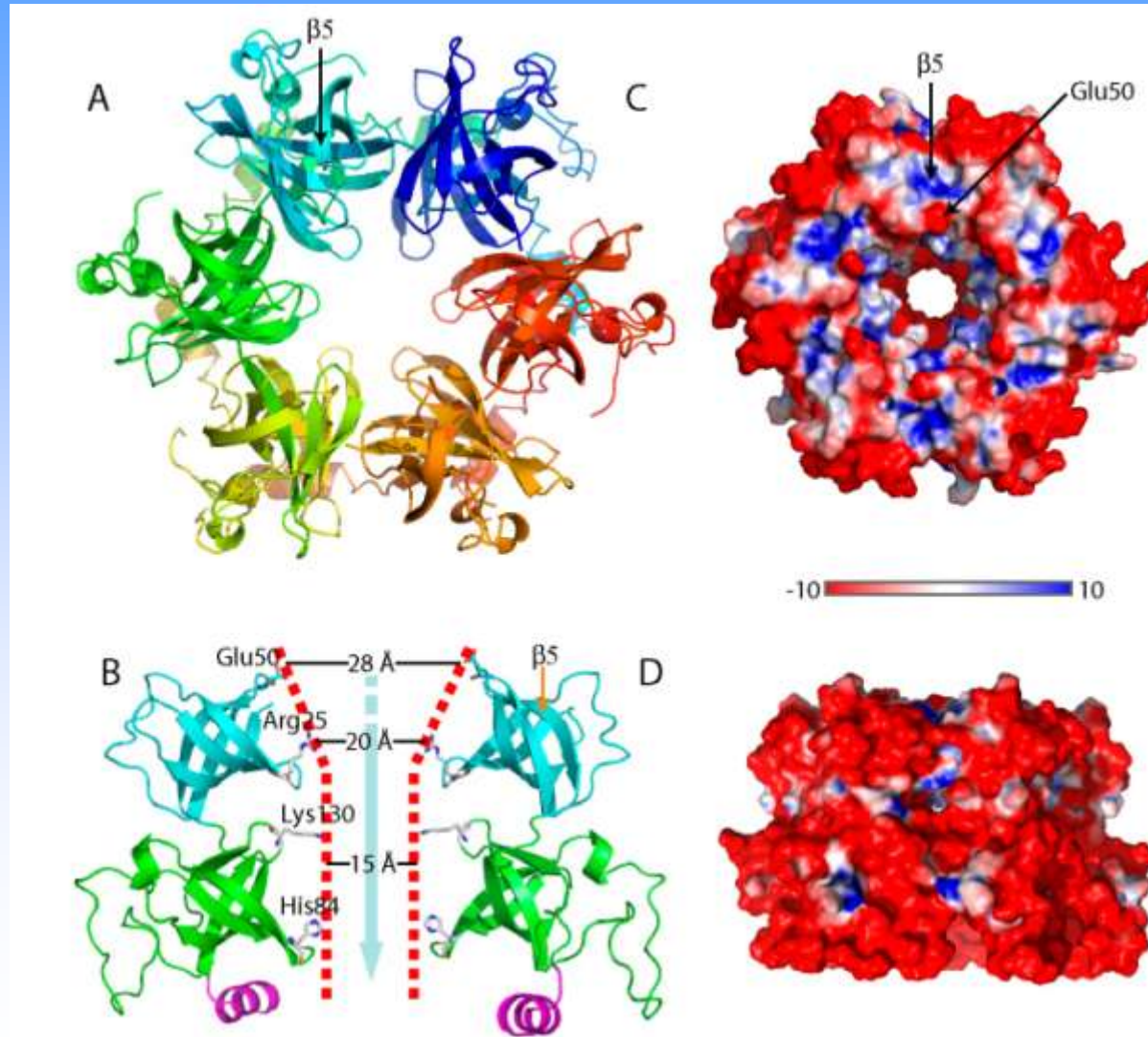
They used both electron microscopy (EM) and x-ray crystallography to determine the structure of the core.



EM pictures show how Mpa interacts with the proteasome

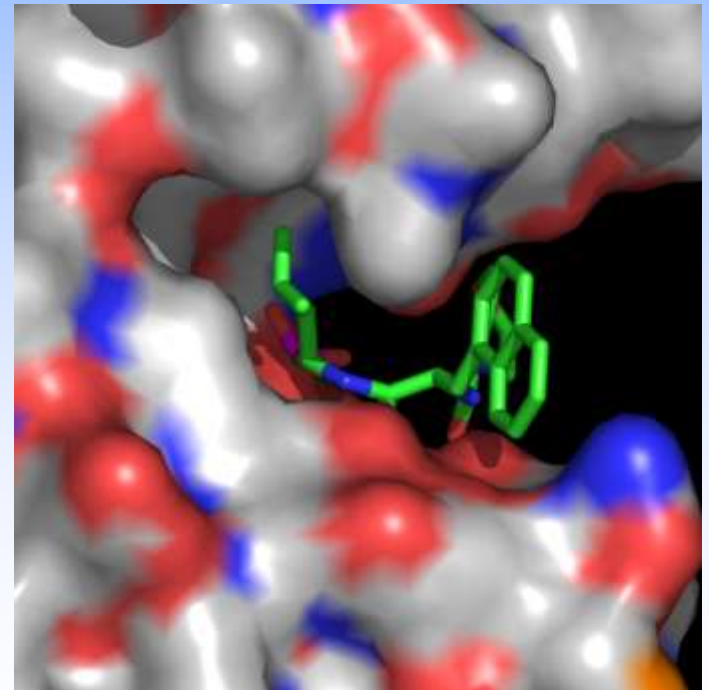
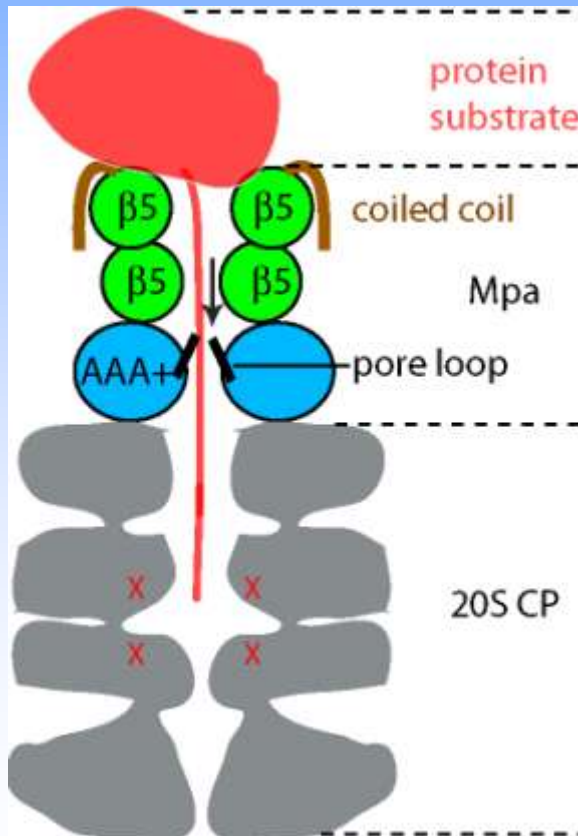


Another crystal structure showed them what part of the Mpa ATPase looks like.



They create a model for how the Mpa ATPase feeds protein for degradation into the Mycobacterium tuberculosis proteasome.

And they also have a drug to defeat the Tb defense, which comes from a cancer drug that inhibits the **human** proteasome.



These studies of the proteasome are examples of the way **Brookhaven Biologists** are using **Biology, Chemistry, Physics, and Math** to answer questions that can be important to human health, to agriculture, and to fundamental science.

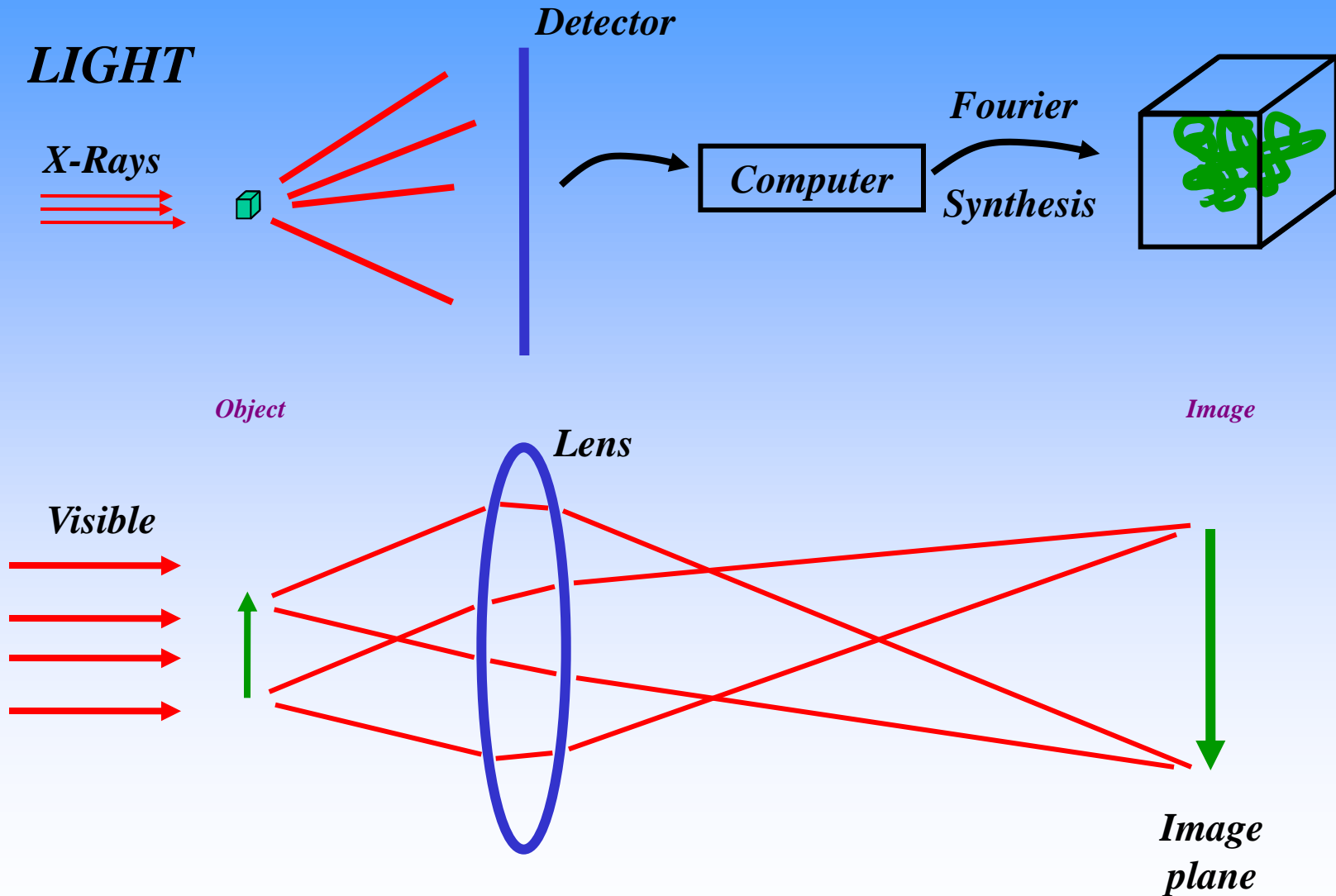
Let's look at how all these disciplines can fit together to provide this wealth of information....

How does it work?

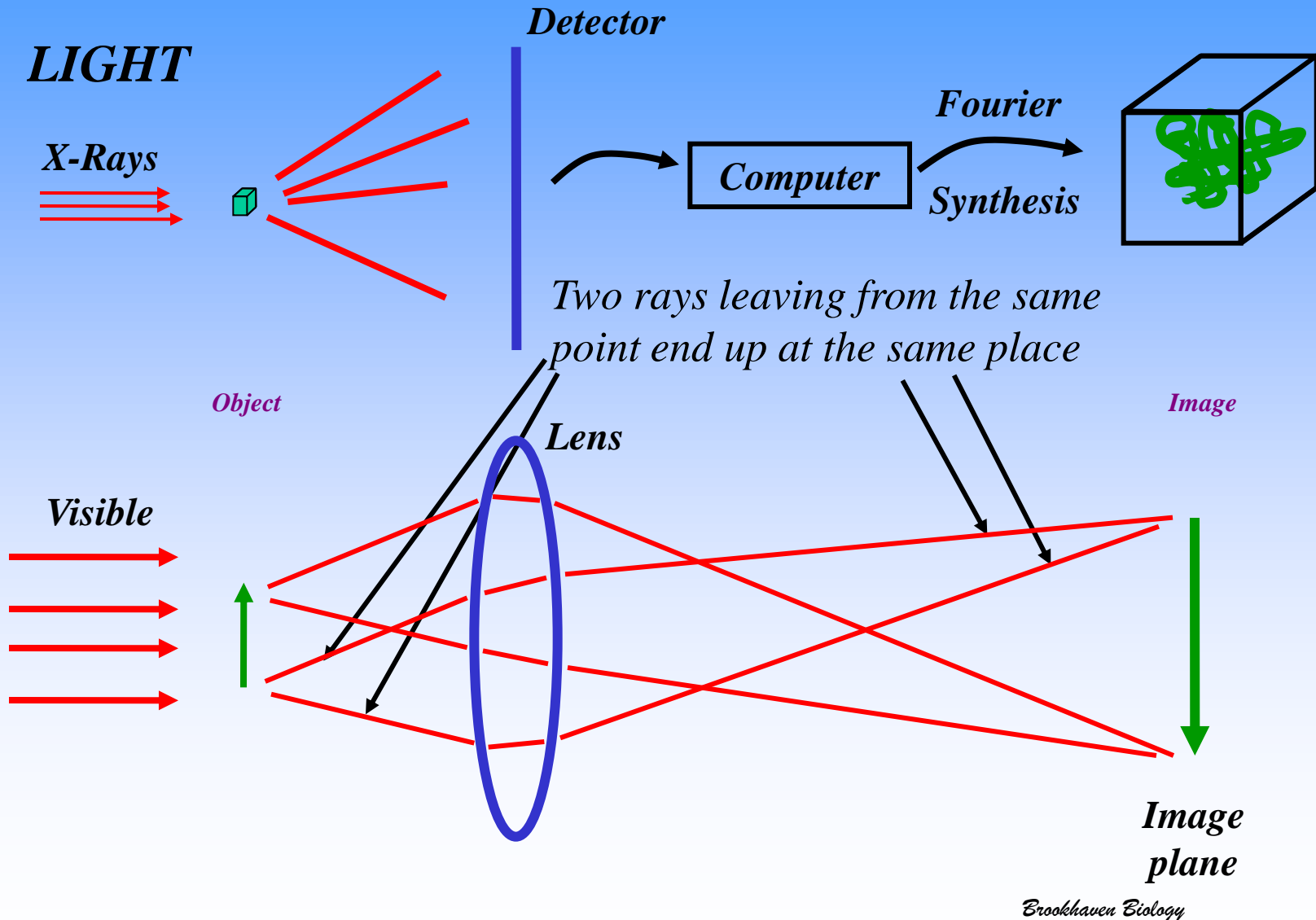
To determine the highest resolution structures, we use **crystals** and **x-rays**. These allow us to “look” at large molecules like the Proteasome.

You’ll be surprised how much you already know about how we might do this.

Creation of a molecule's image from a crystal has similarities to creating an image with a lens

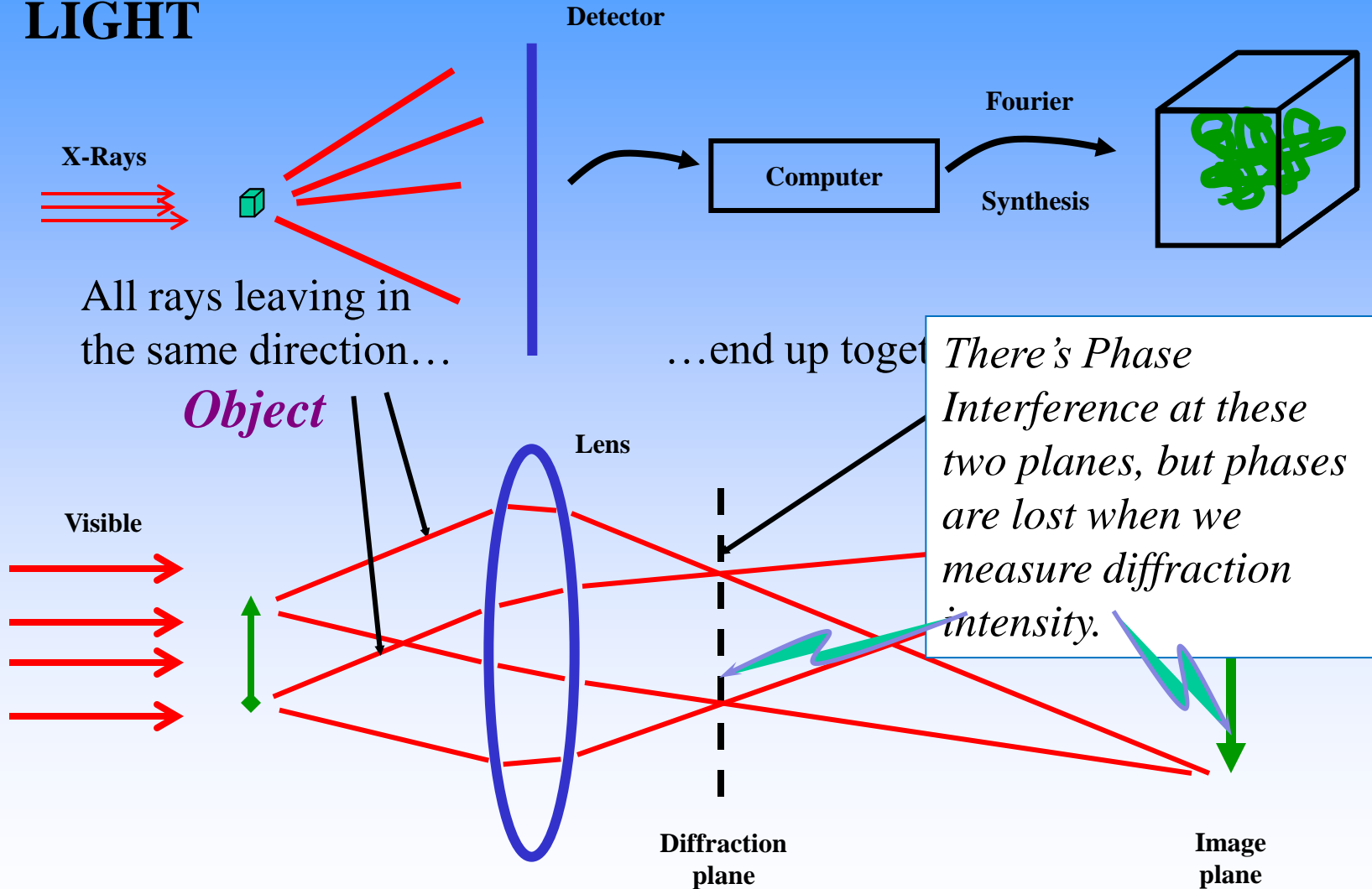


You already understand a little about how lenses work

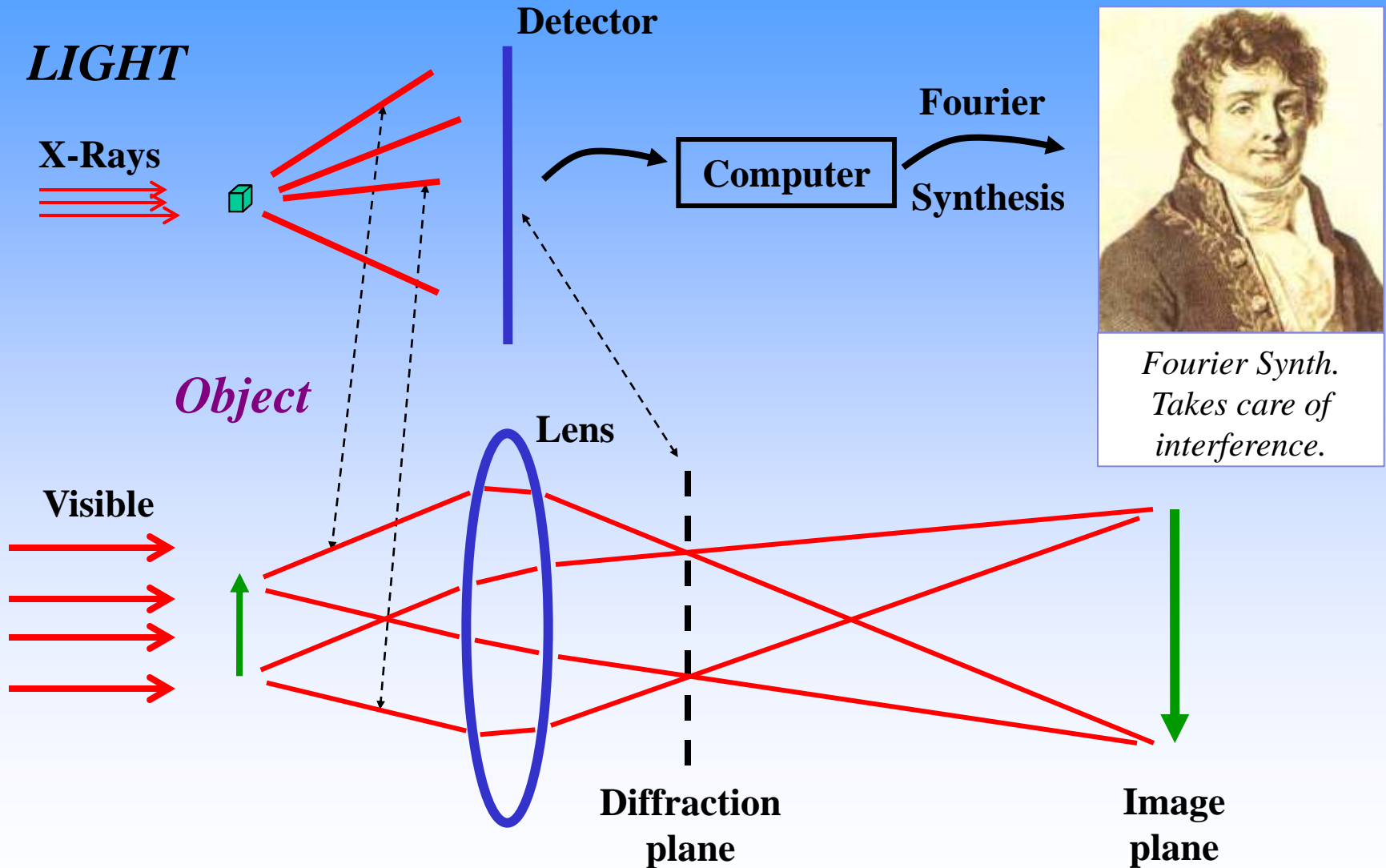


Maybe you didn't know ...

LIGHT



We use a crystal to give us diffraction



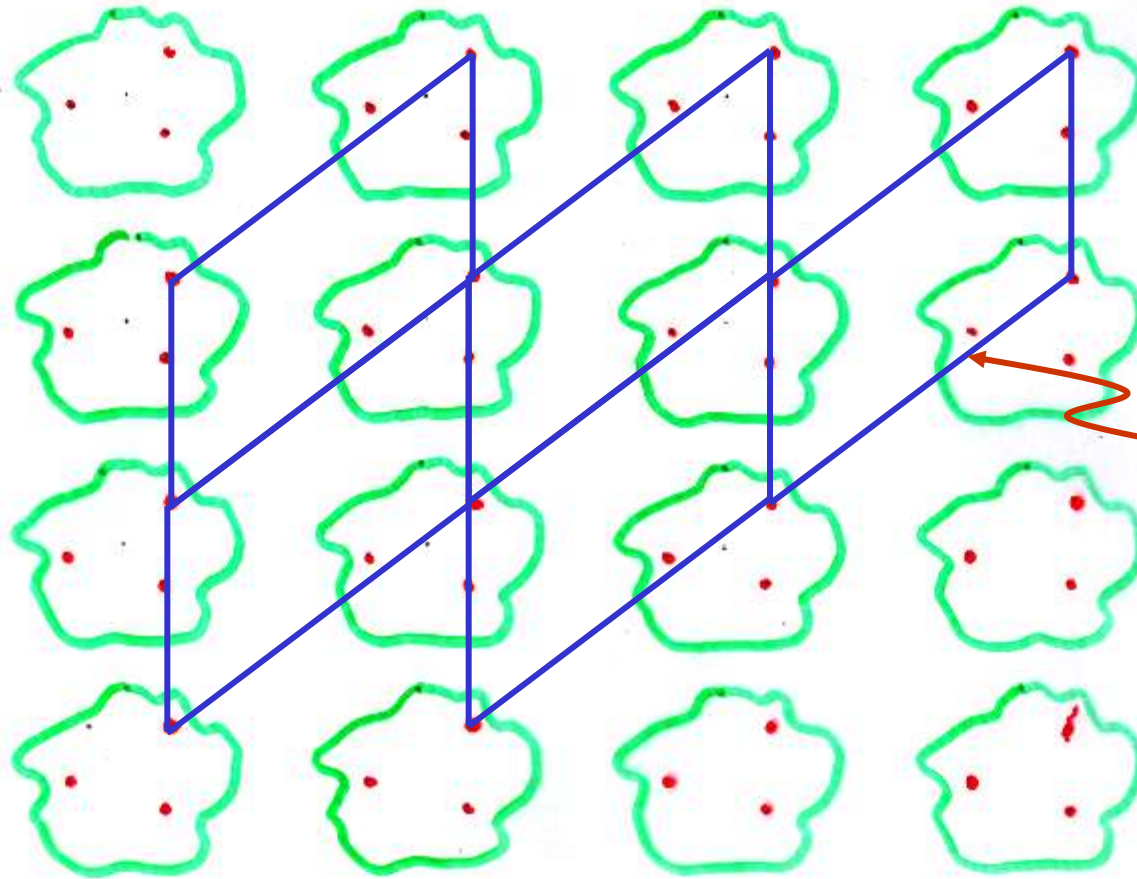
Why do we use **x-rays**?

- The features we're trying to see are on the order of the **distance between atoms: 10^{-10} meters**.
- To “see” the atoms, we need to use light with a **wavelength** that is near to **this distance**.
- X-Rays (x-ray light) have a **suitable wavelength**
- The x-rays are scattered by the electrons on the atoms so what we **see** is the **electrons**.

What is a **crystal**?

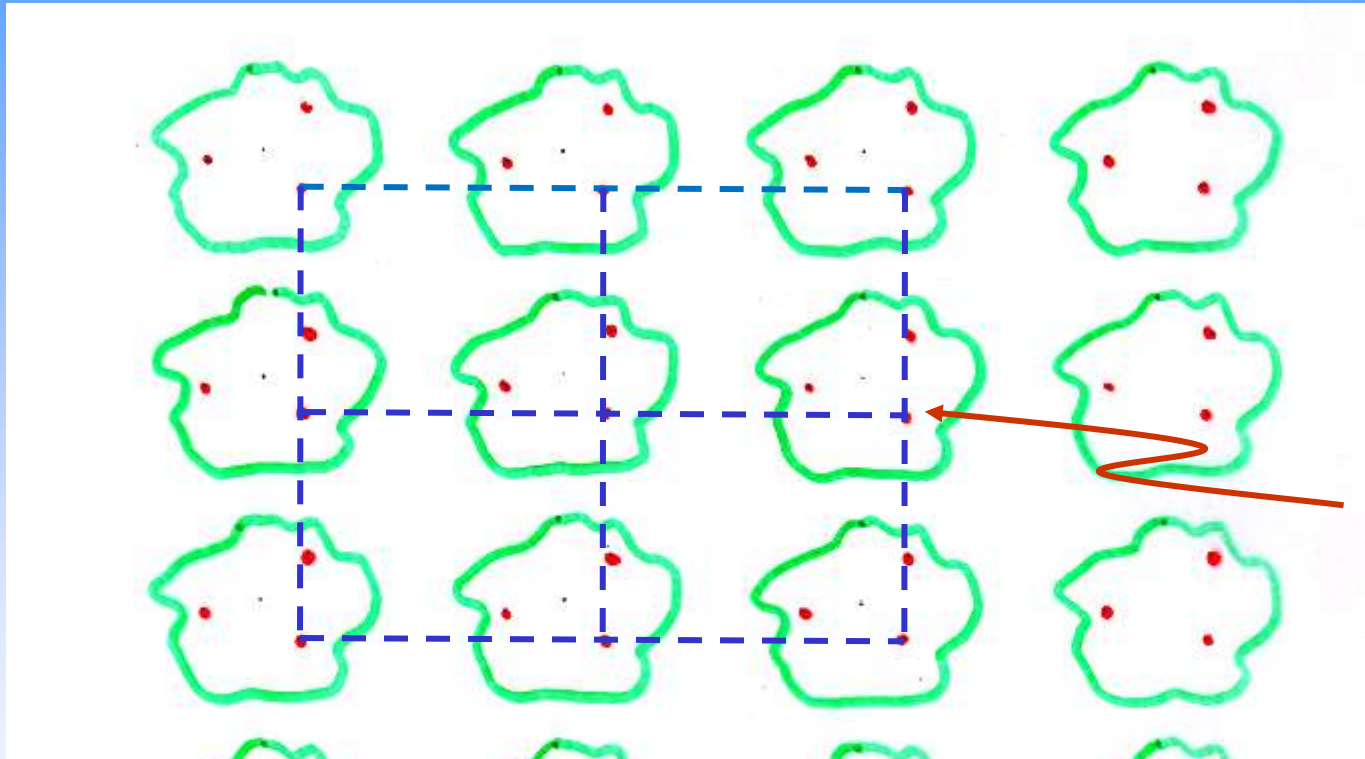
- A crystal is a **periodic arrangement** of objects (molecules) repeating in two or three dimensions.
- The **repeating unit** is a parallelepiped (three-dimensional) or a parallelogram (two-dimensional).
- A crystal of the proteosome will be a tenth of a mm on a side and contain **10^{15} molecules**.

Here's one choice of repeating unit in this crystal made of apple trees



Parallelograms
defining crystal
repeat.

We could make a different choice of repeating unit



Other
parallelograms
defining crystal
repeat.

*In both cases the repeating unit (**Unit Cell**) has the same AREA, or VOLUME for a three-dimensional crystal.*

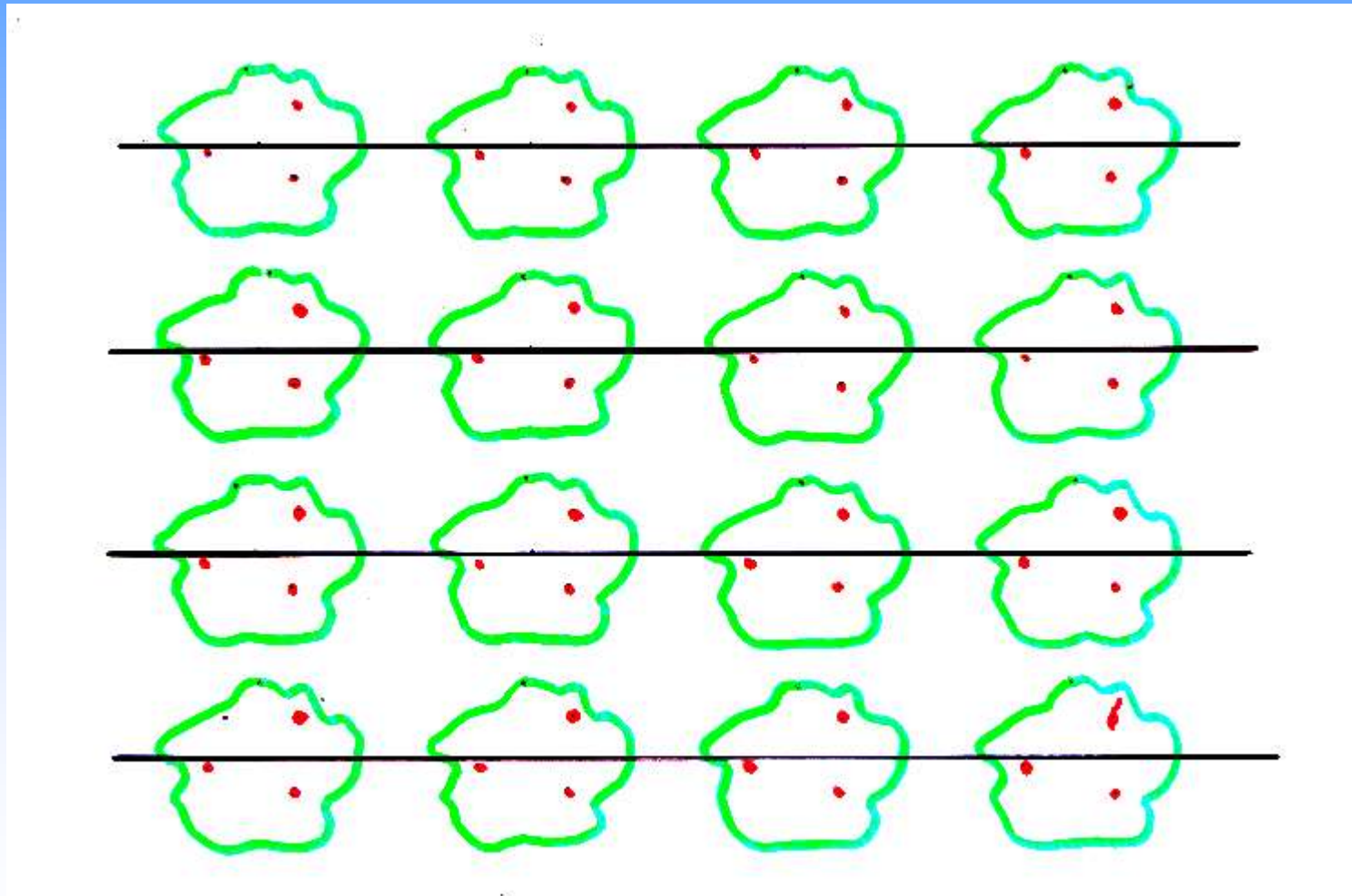
Why do we use crystals when we'd like to see one molecule?

- We can't **focus** enough x-rays into a small enough volume to “see” a molecule. We use lots of molecules in a **crystal** to get a bigger target.
- Even if we could, the x-rays would **burn up** the molecule.
- Even if that would work, we **don't have a lens** for the x-rays.
- The crystal **amplifies the signal**, and gives us a way to get the **phase information** back.

Let's return to our crystal made of apple trees, and define “planes” in that crystal.

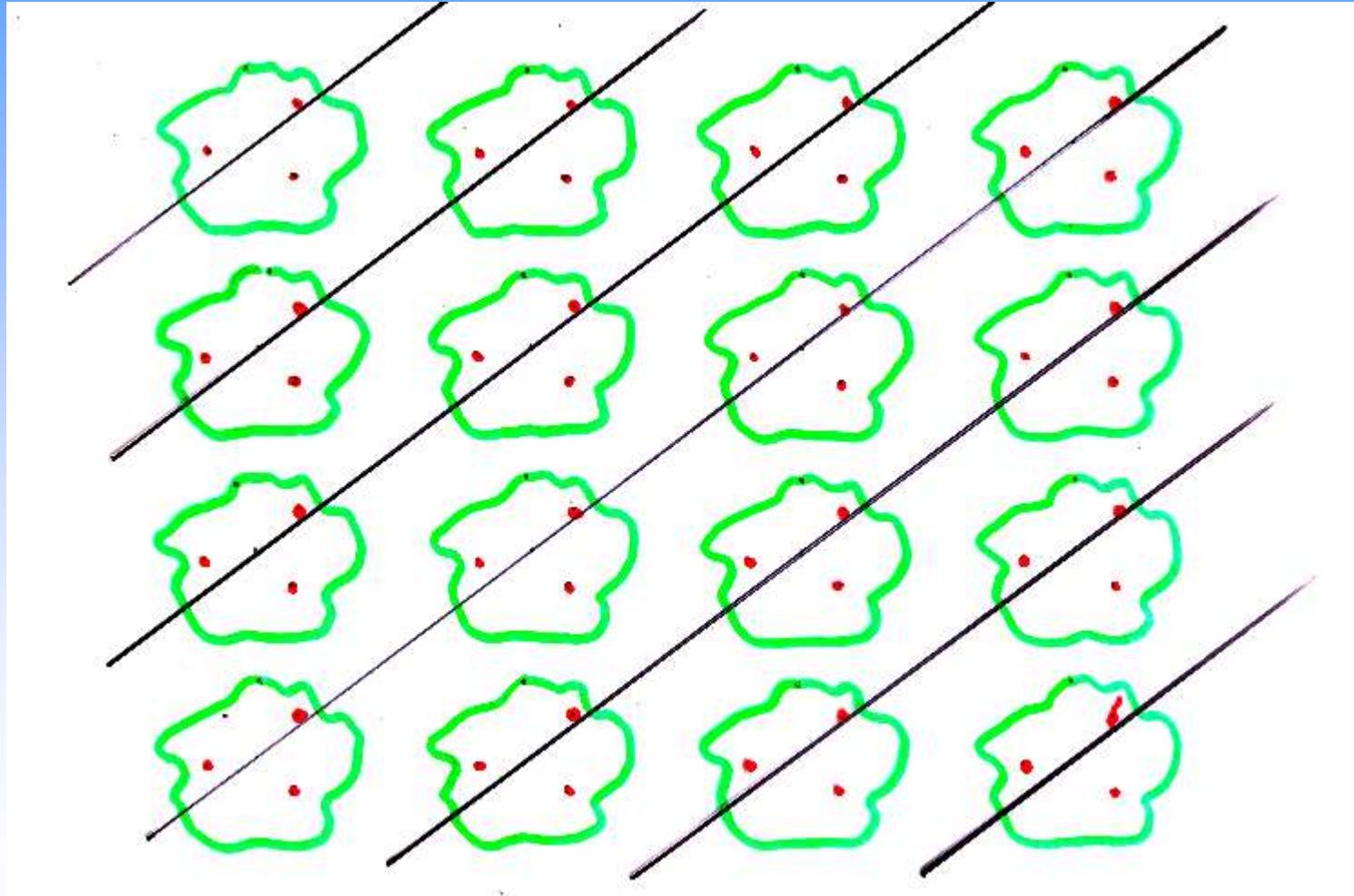


We can slice the crystal at **lattice points**:
all planes pass through the same apple

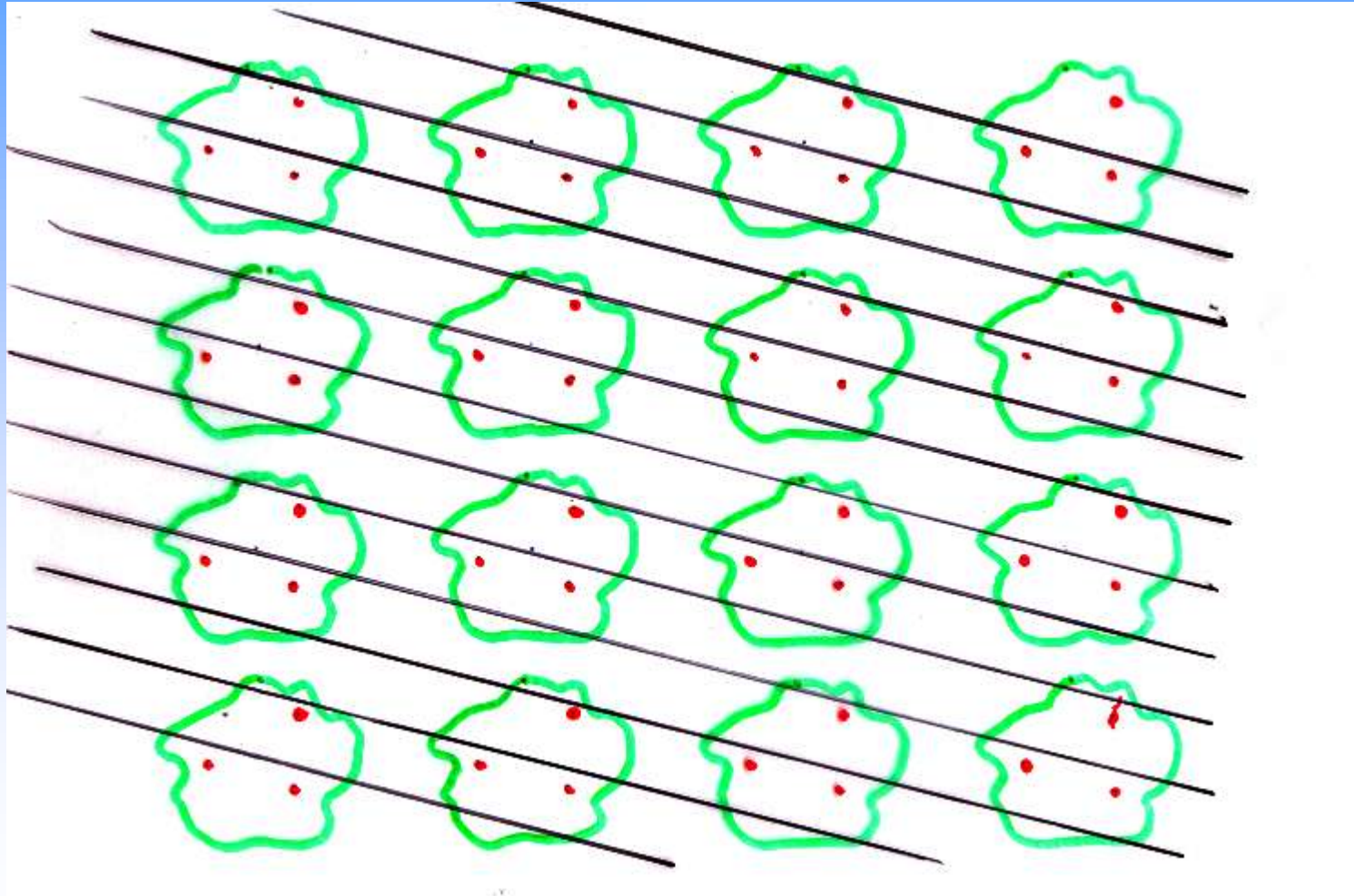


And at other angles. Notice:

- planes all pass by the same apple;
- the “stuff” between pairs of planes is always the same.

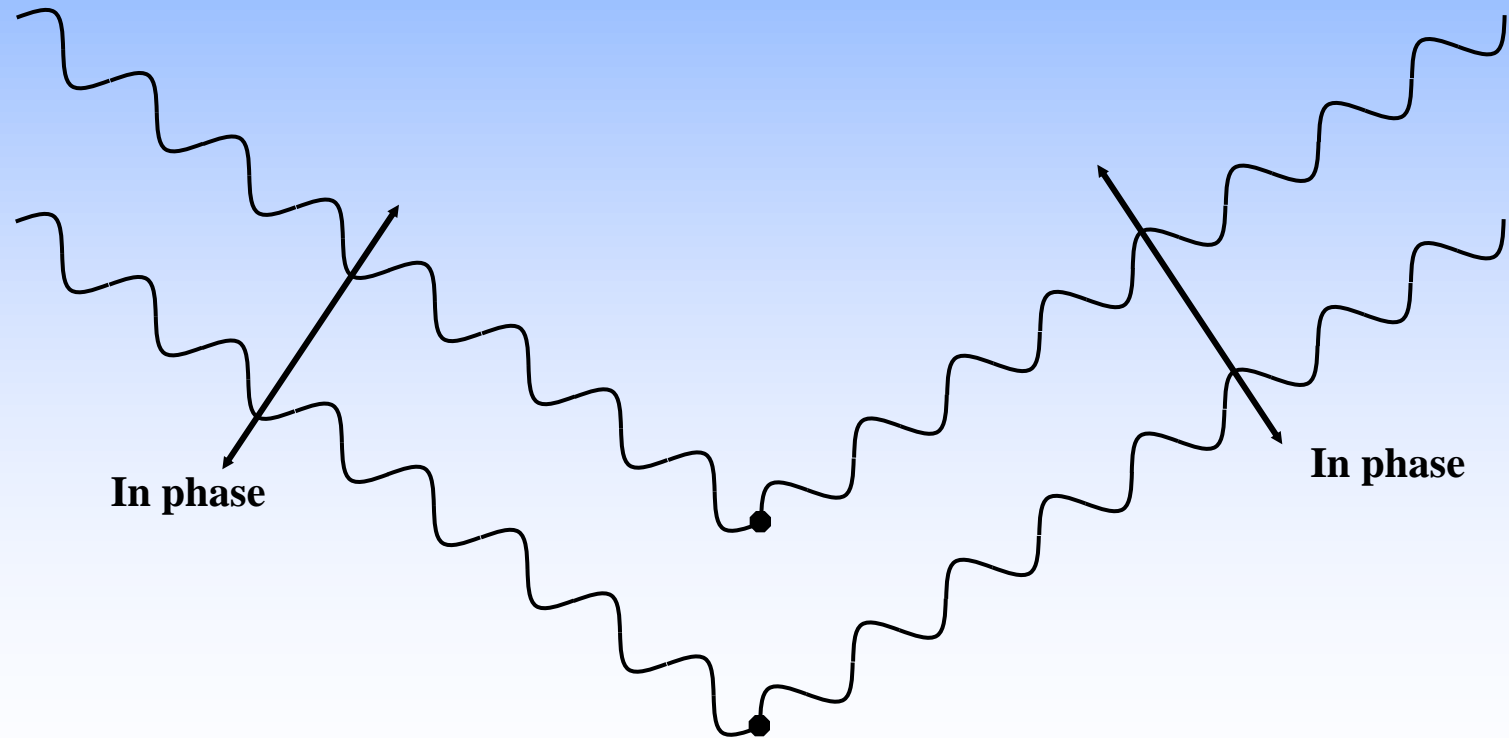


And one more time...

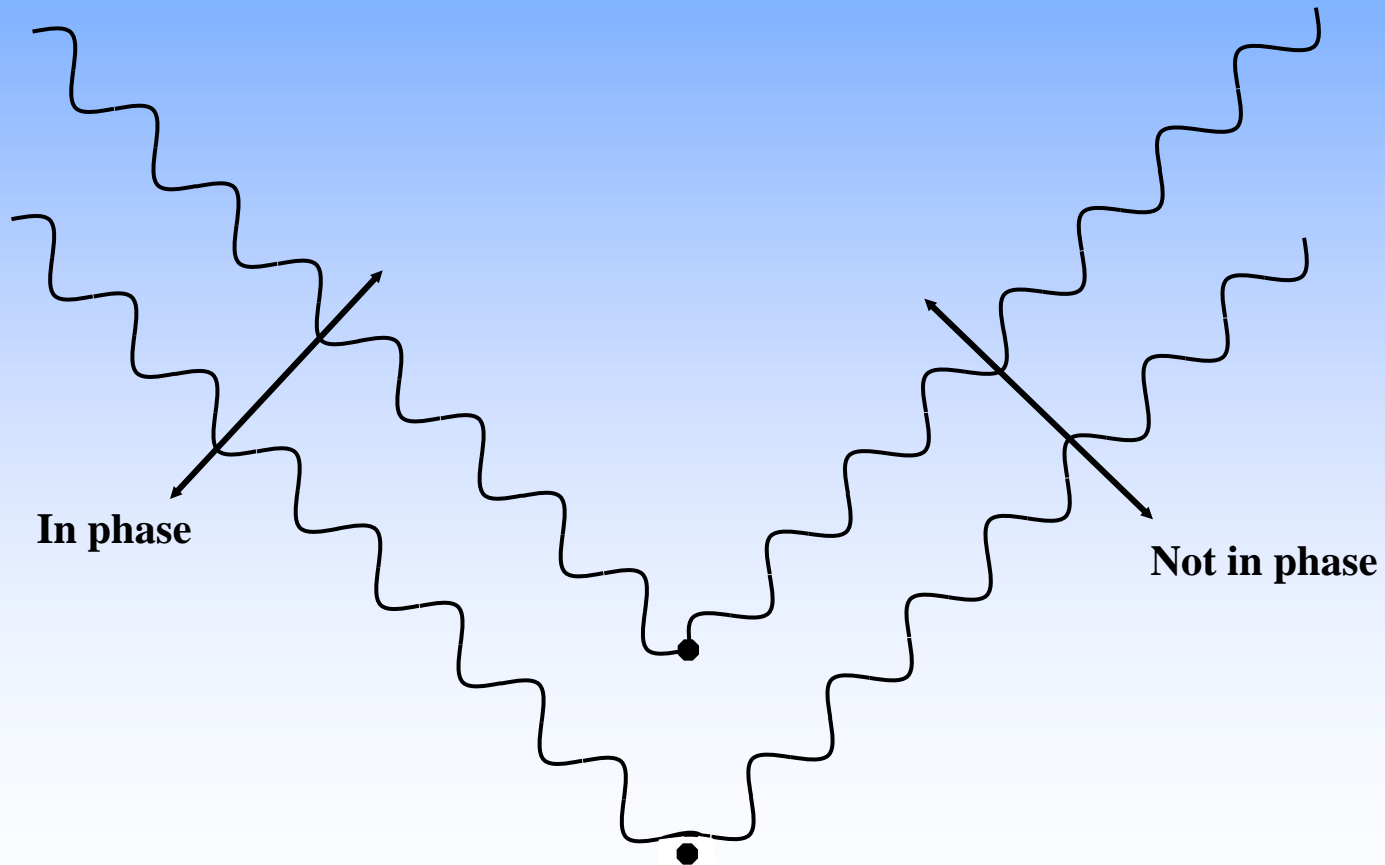


Diffraction – Let's do a thought experiment.

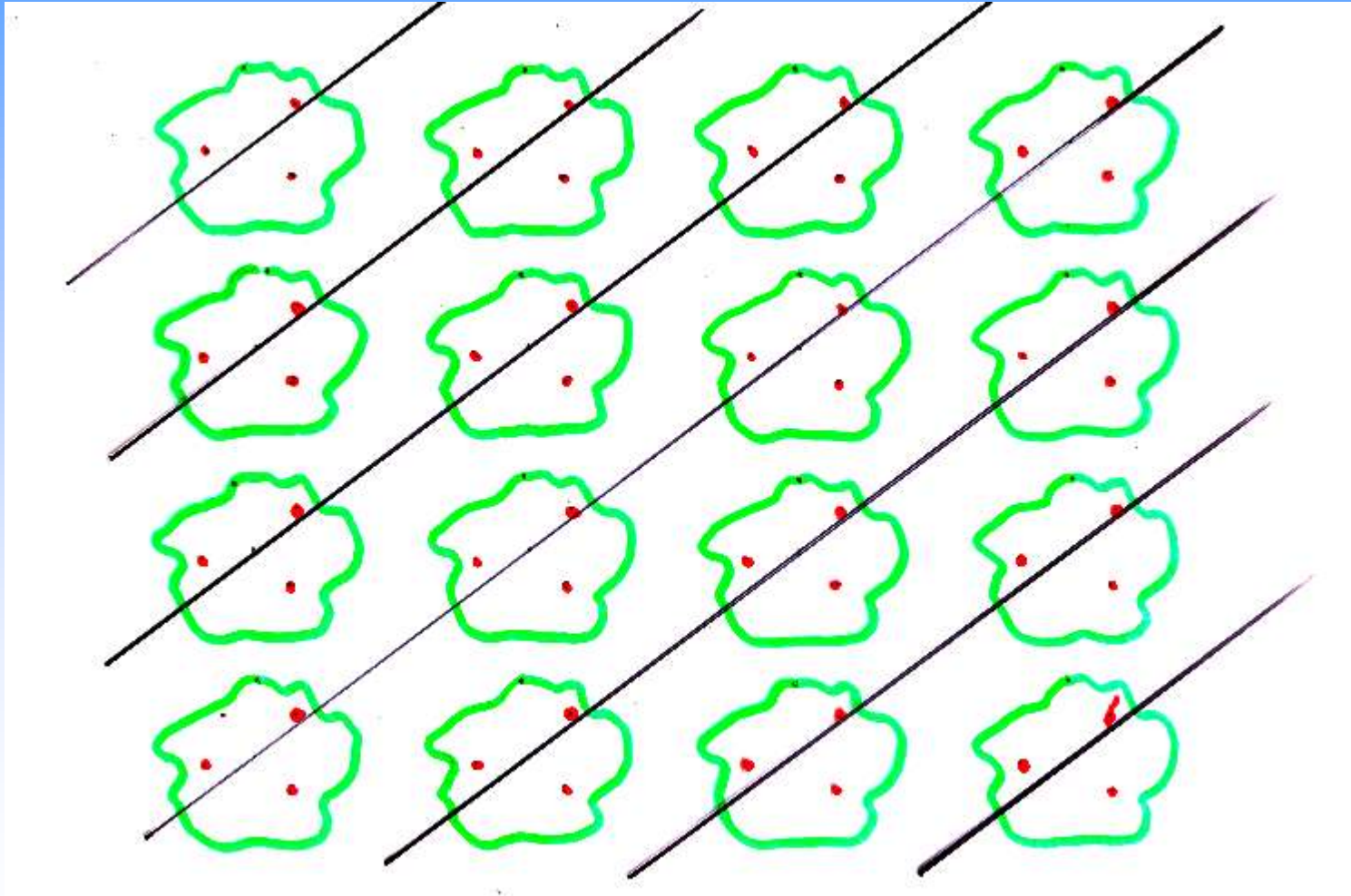
- Think of the material between the lattice planes as just two atoms, suspended in space.
- Send a beam of x-rays at these atoms.
- If the angle is just right for the wavelength and distance between the atoms, the scattered x-rays will be in phase, and they will interfere constructively.

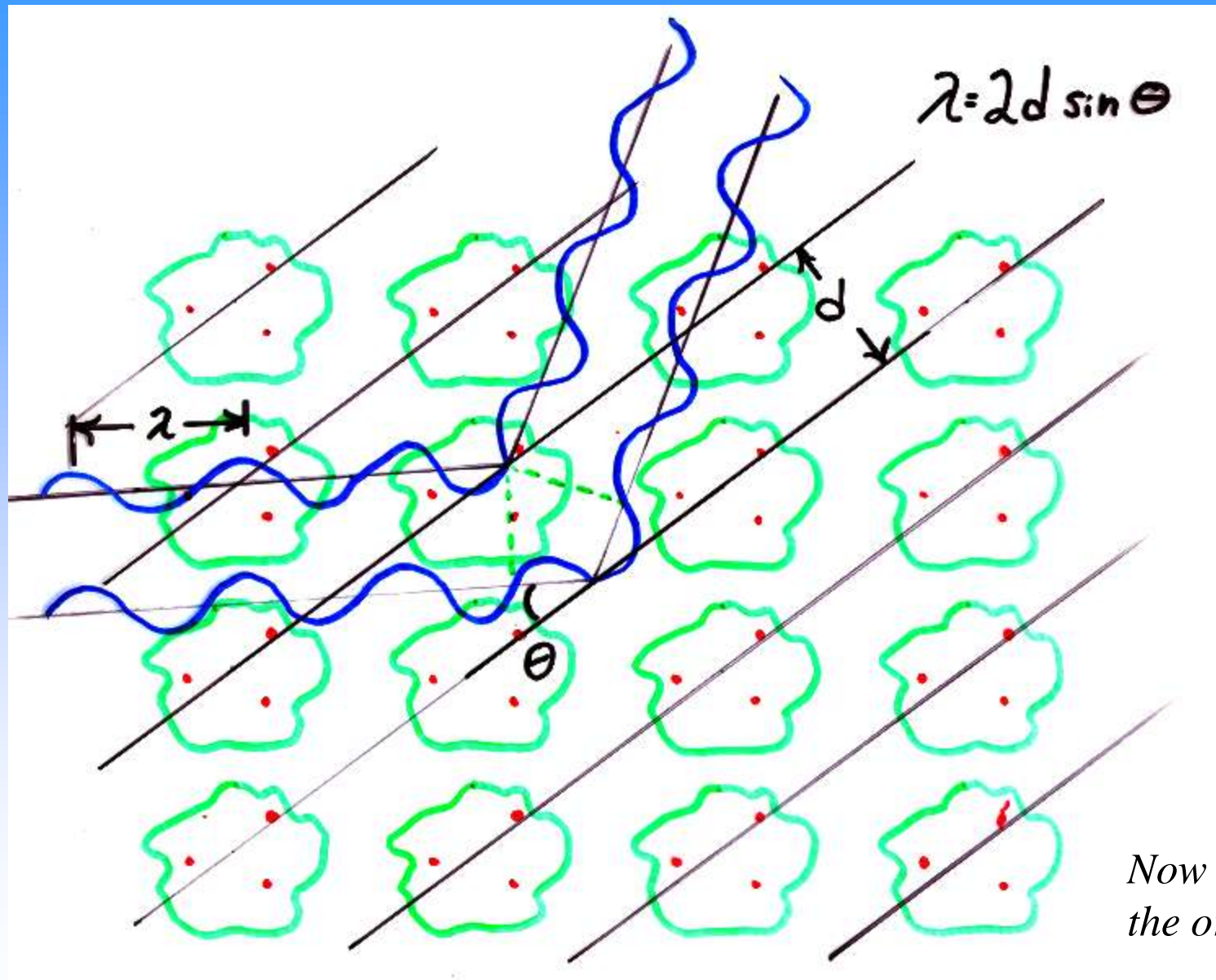


**On the other hand, if things are not right,
they won't be in phase, and there will be no
constructive interference, no diffraction.**



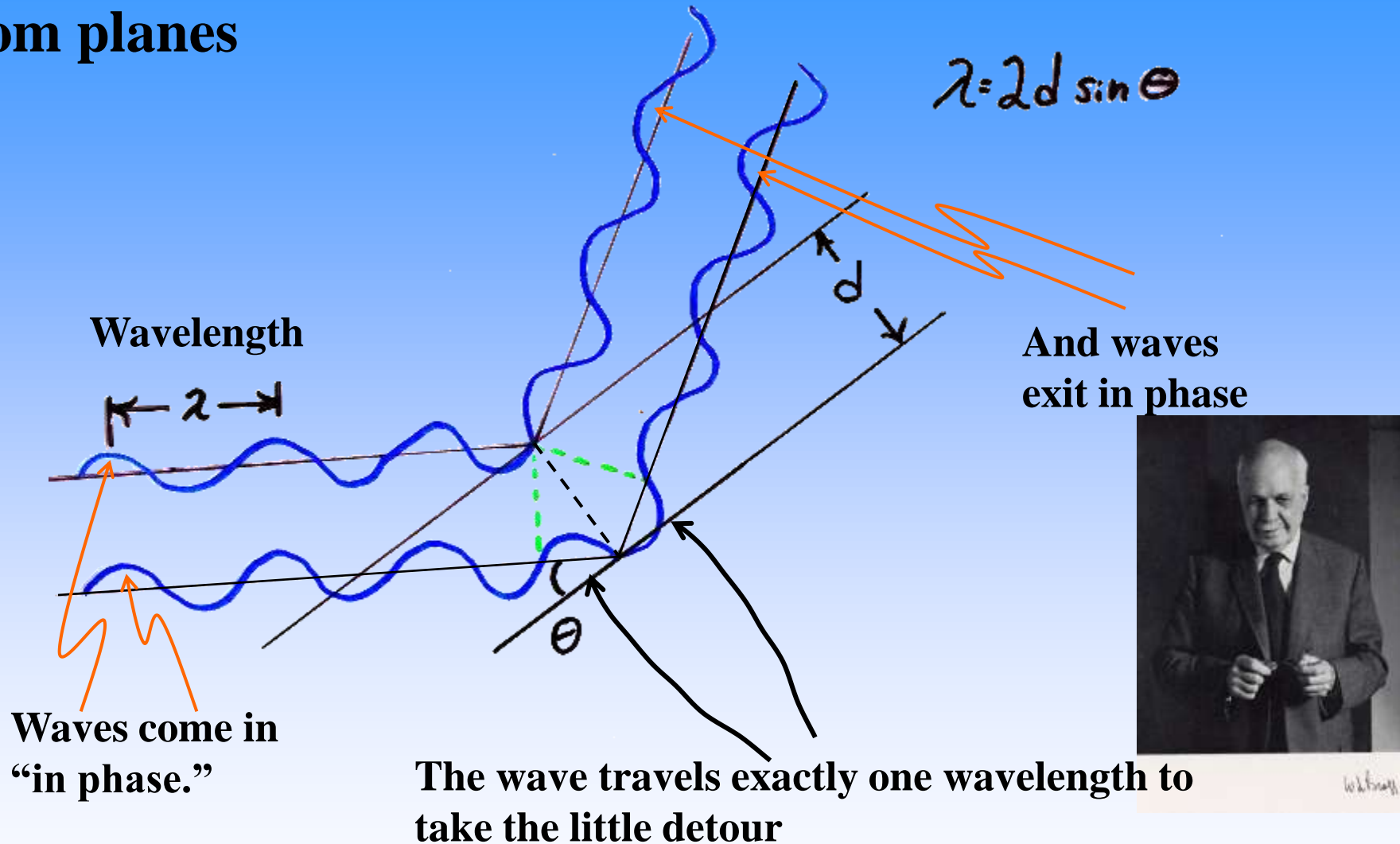
Now, as we think of the stuff between the lattice planes as being like those two atoms, we try to write a law that will show conditions to get diffraction.





*Now get rid of
the orchard...*

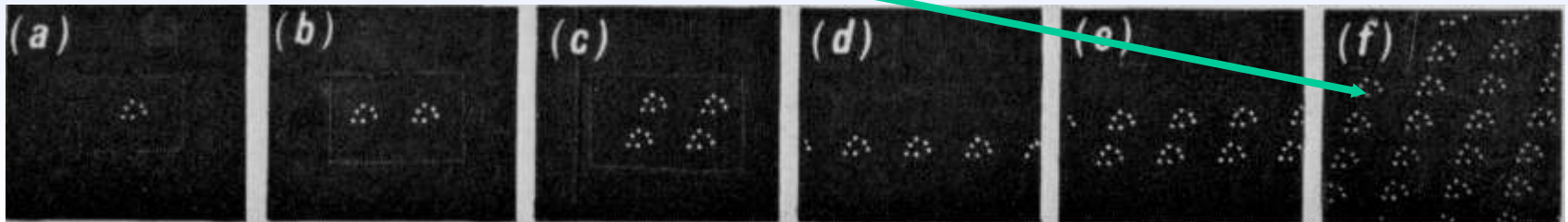
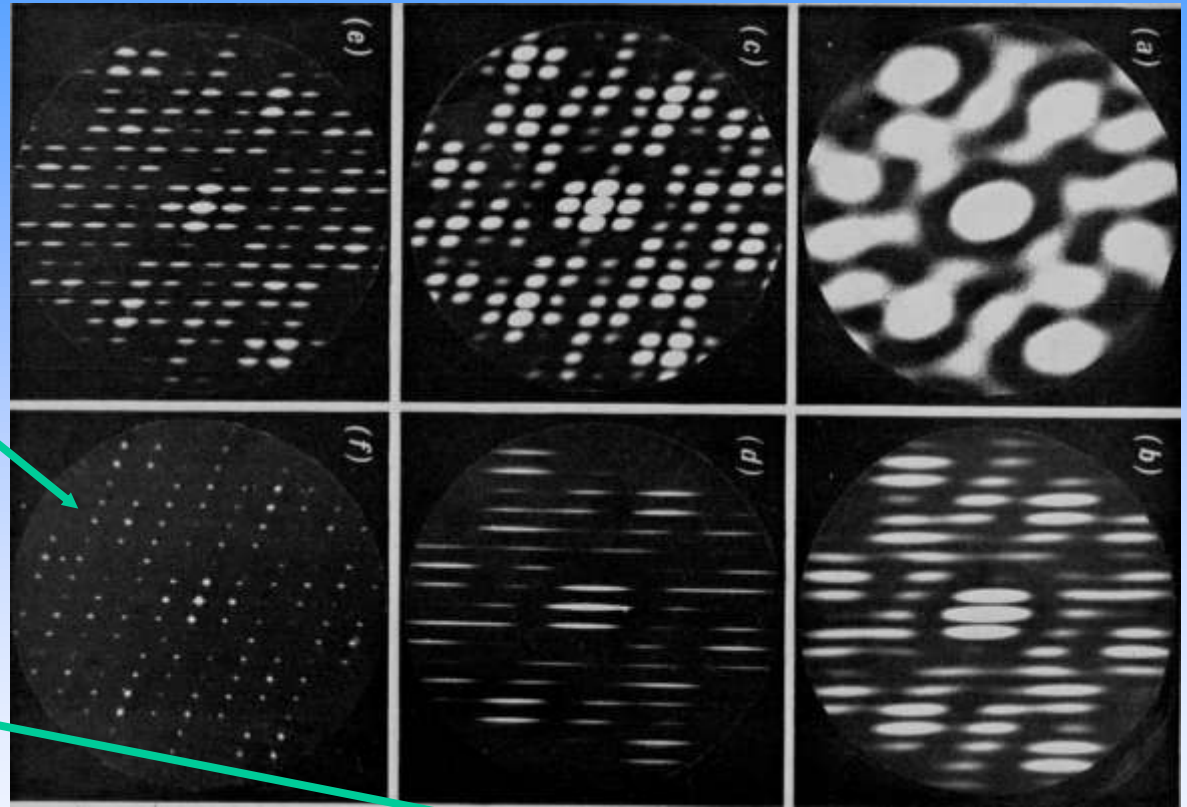
Bragg's Law describes diffraction as *if it were* reflection from planes



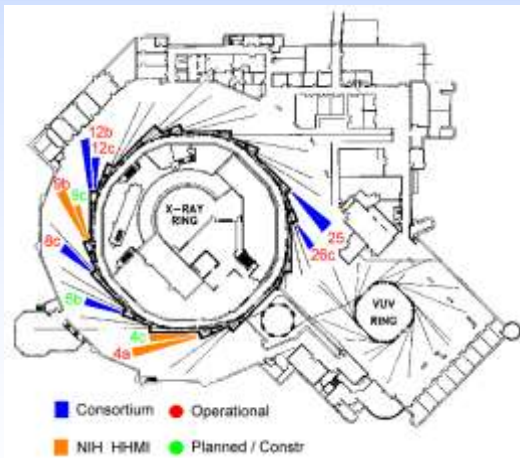
W. Bragg

Here's a two-dimensional example where we build up a crystal with a six-atom molecule and examine the diffraction pattern.

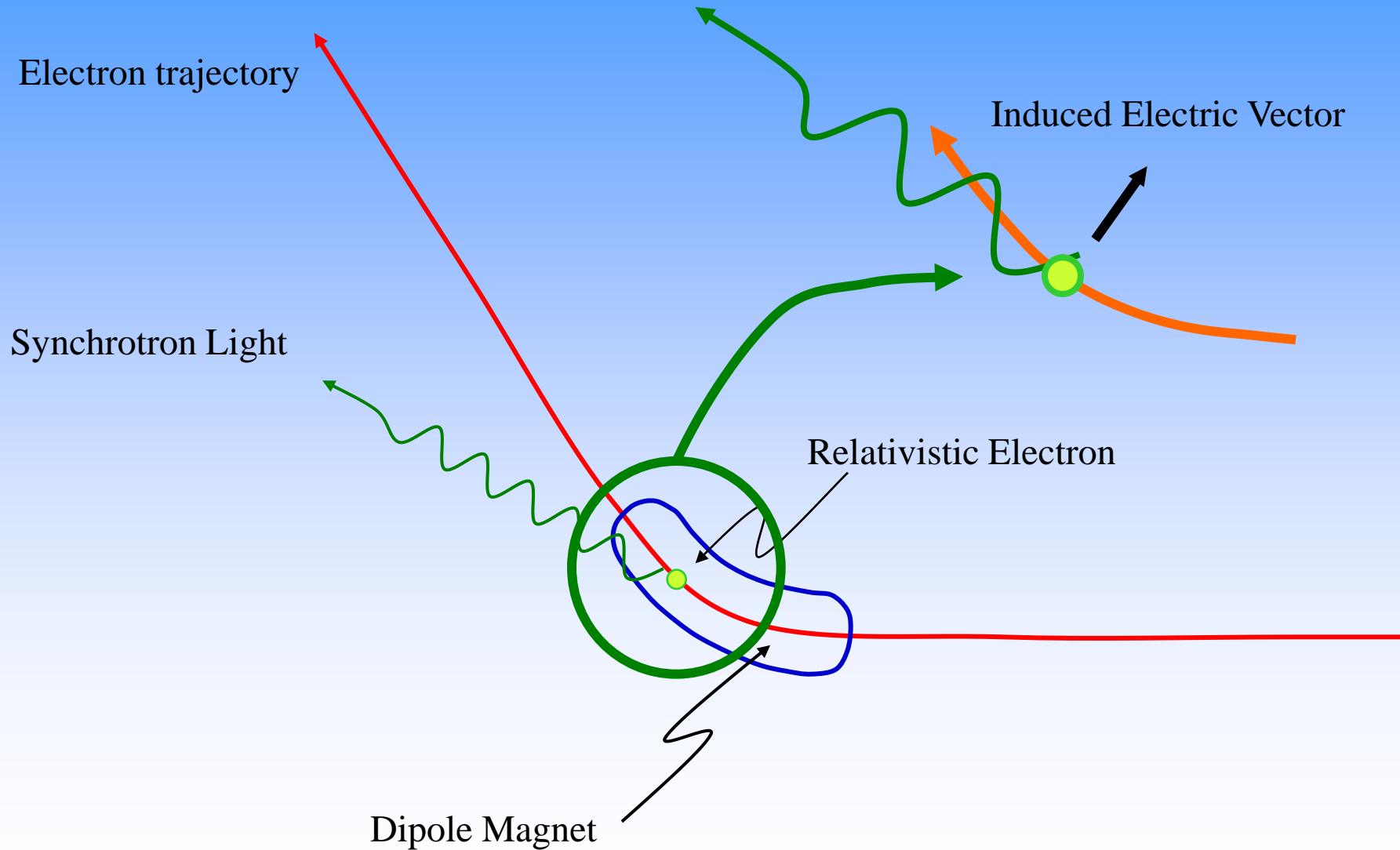
Each spot represents the intensity of reflection from one set of planes cutting through the crystal.



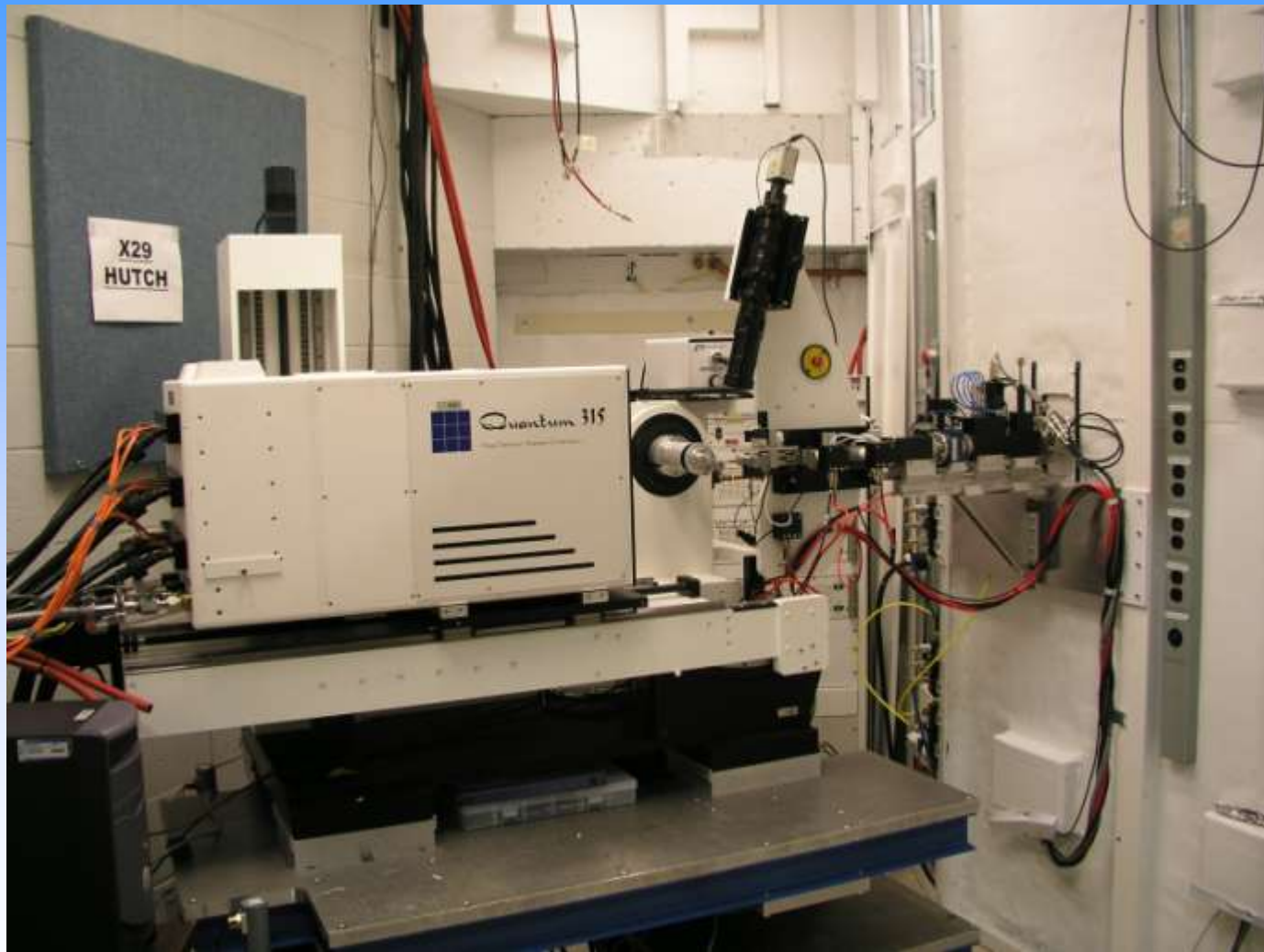
Brookhaven Scientists use **X-Rays** from the **National Synchrotron Light Source** and lots of computing power to determine these structures

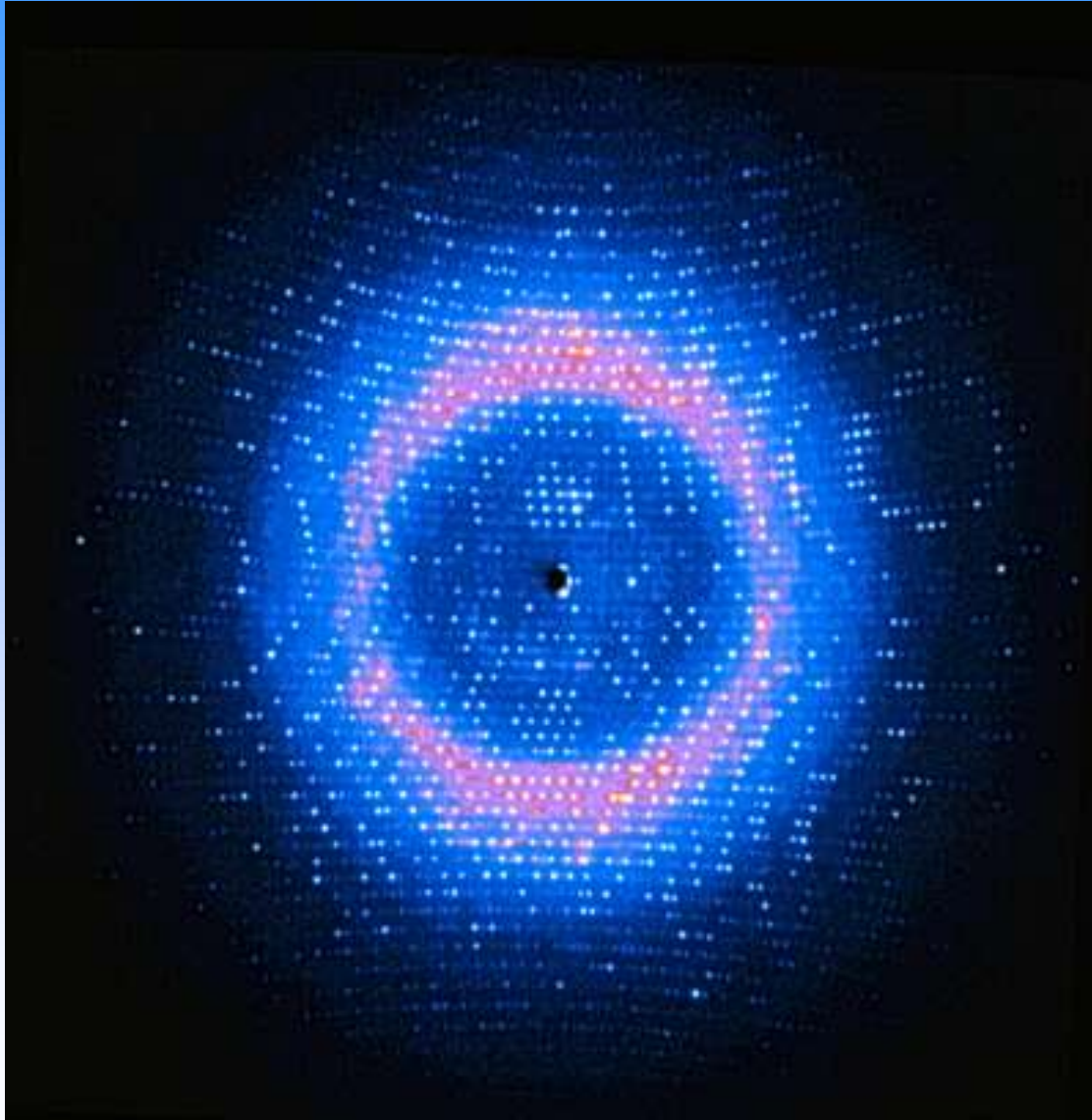


The Source of Synchrotron Radiation



Complex apparatus allows us to measure diffraction patterns from protein crystals

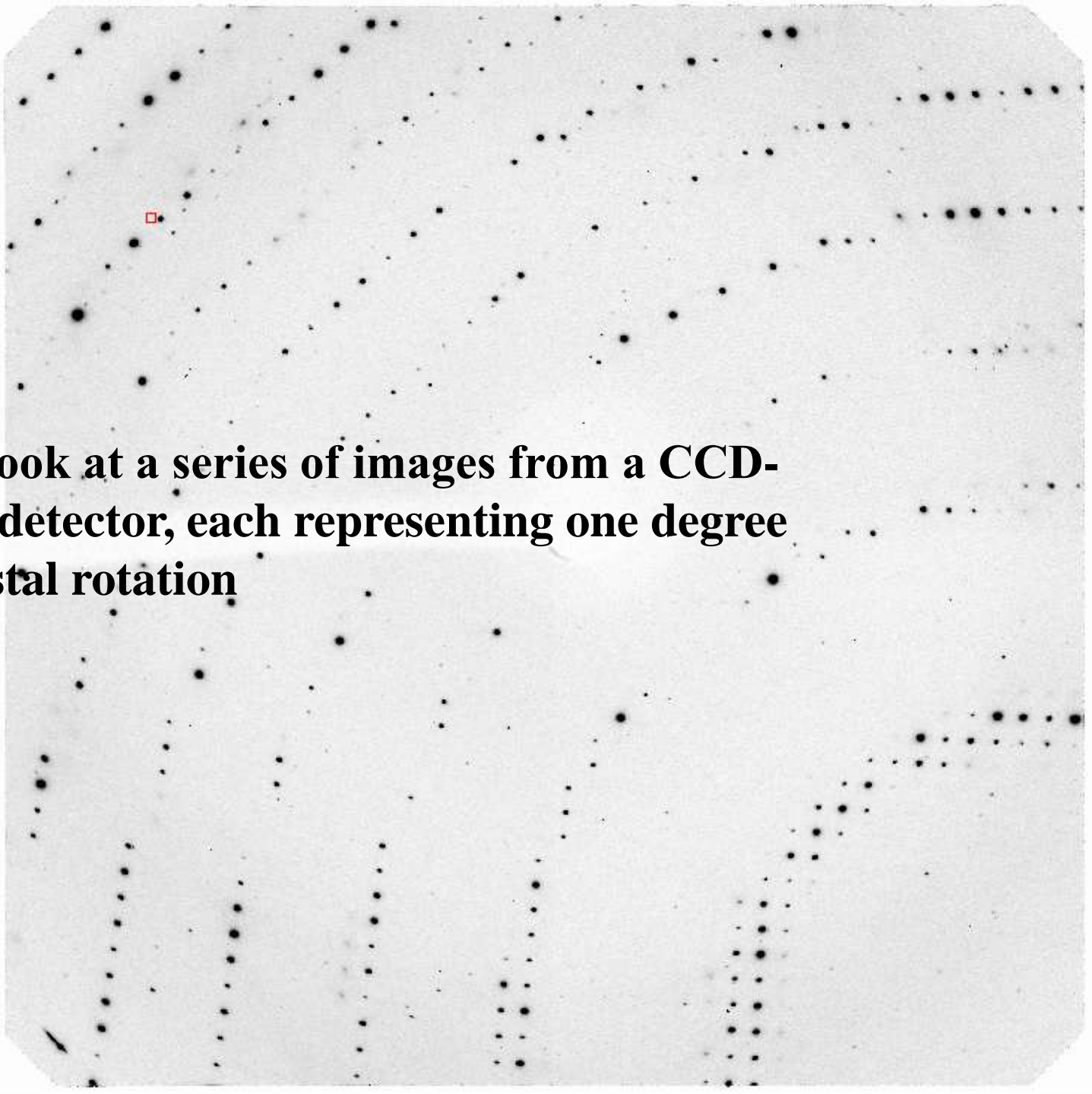


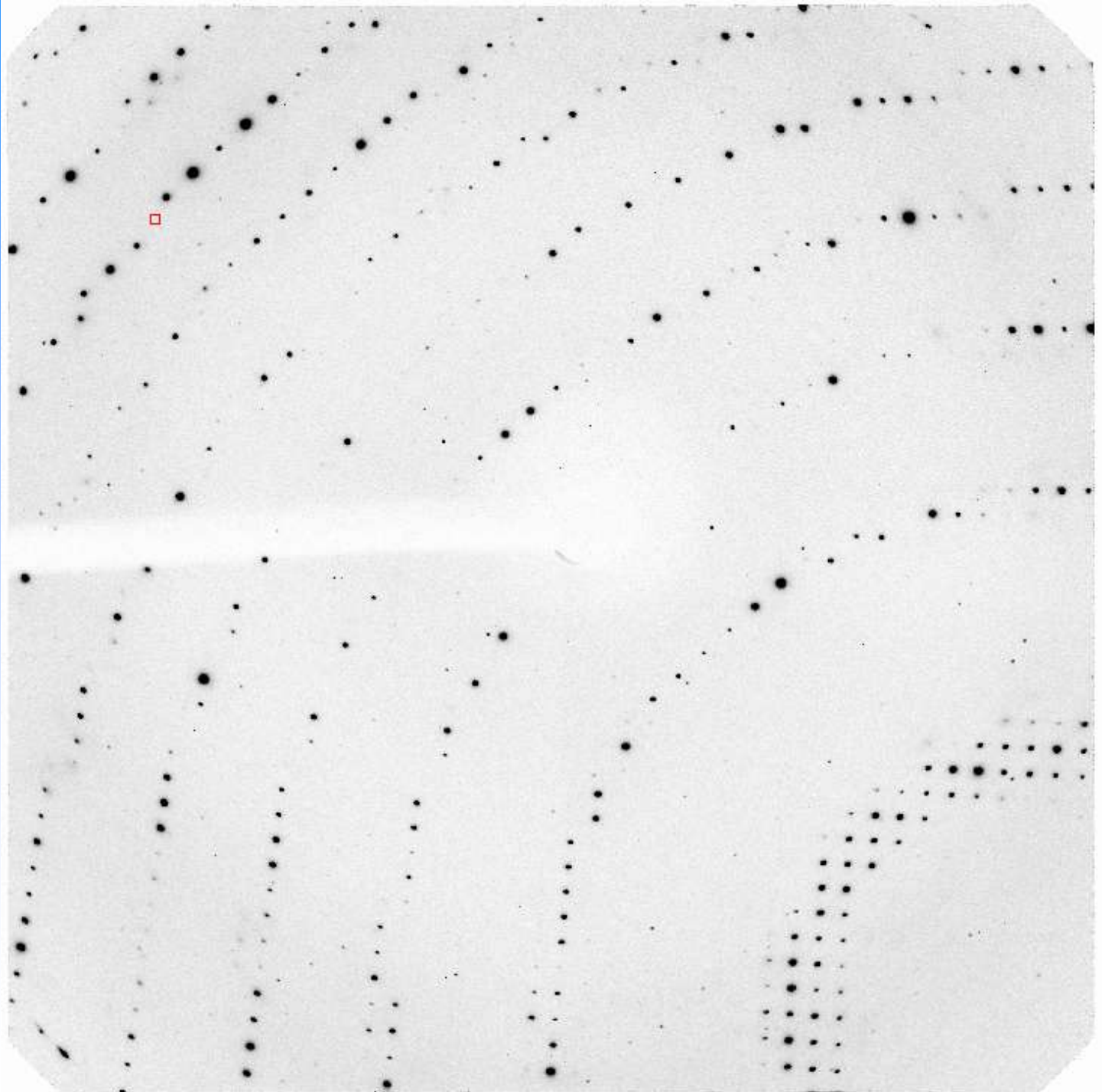


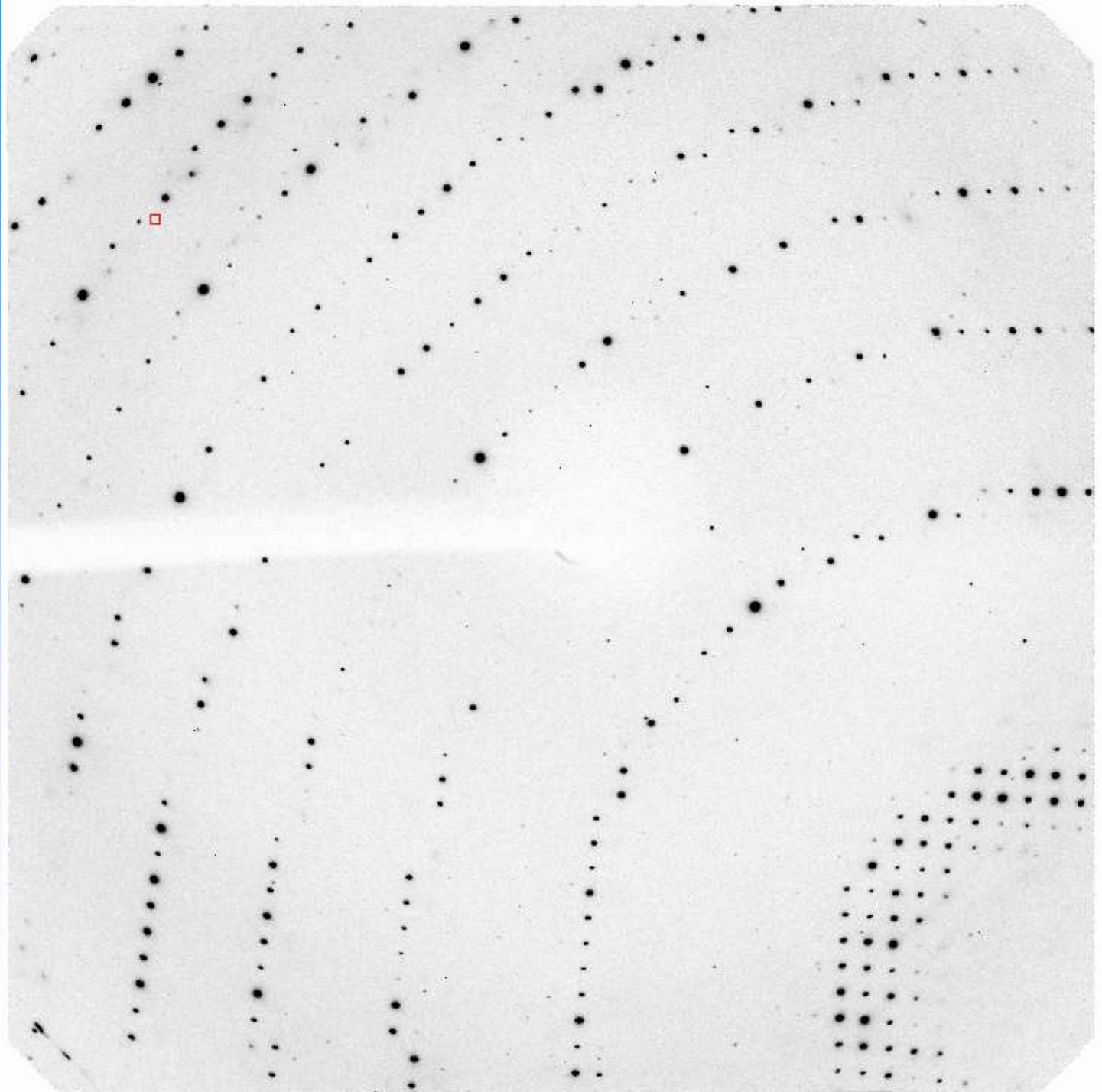
Simple rotation geometry produces a complicated pattern that requires good software to interpret. Modern CCD-based detectors with four-circle diffractometers record such patterns and measure every spot intensity.

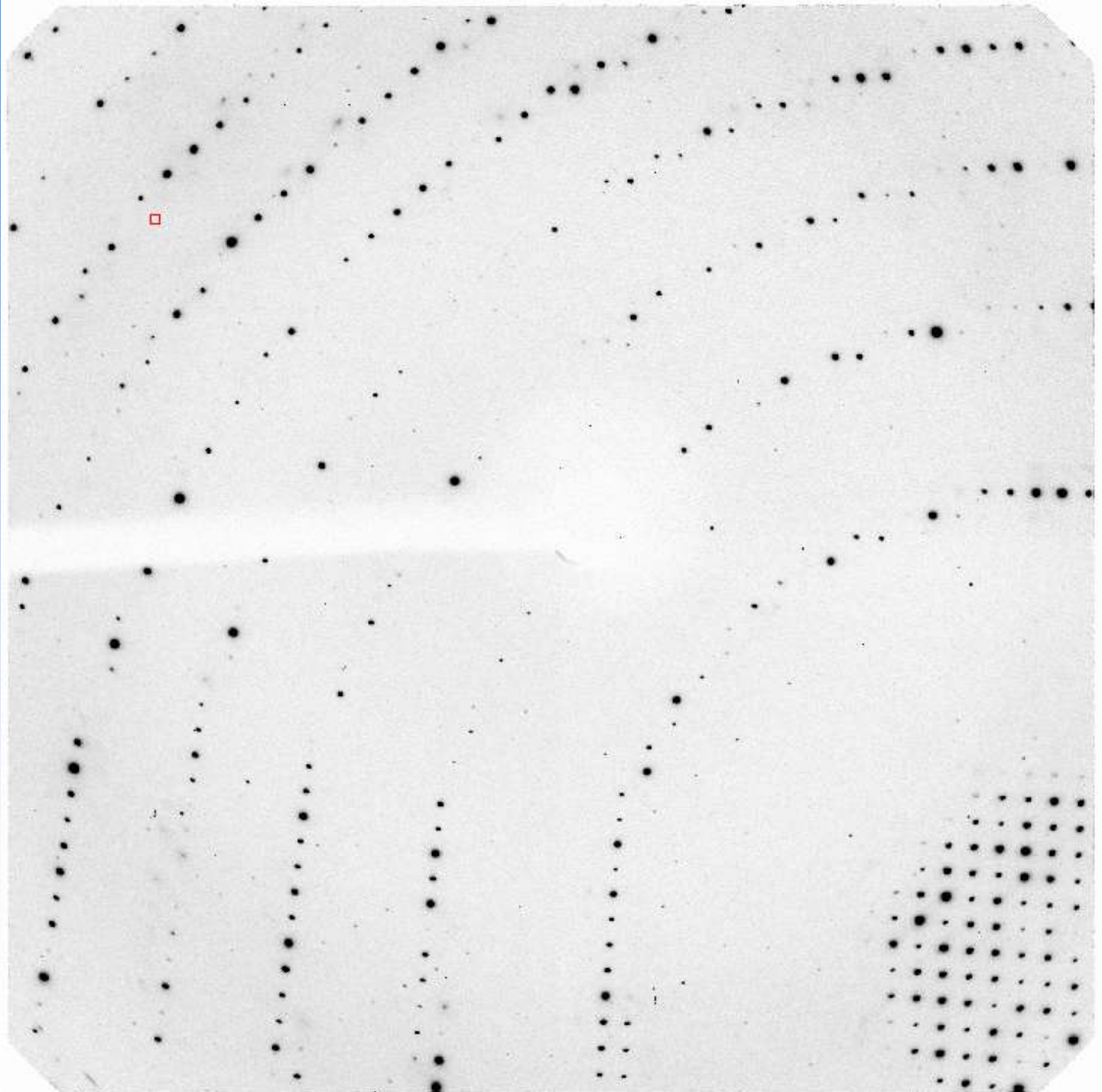
An antique rotation photograph of B-Phyocerythrin -- real x-ray film.

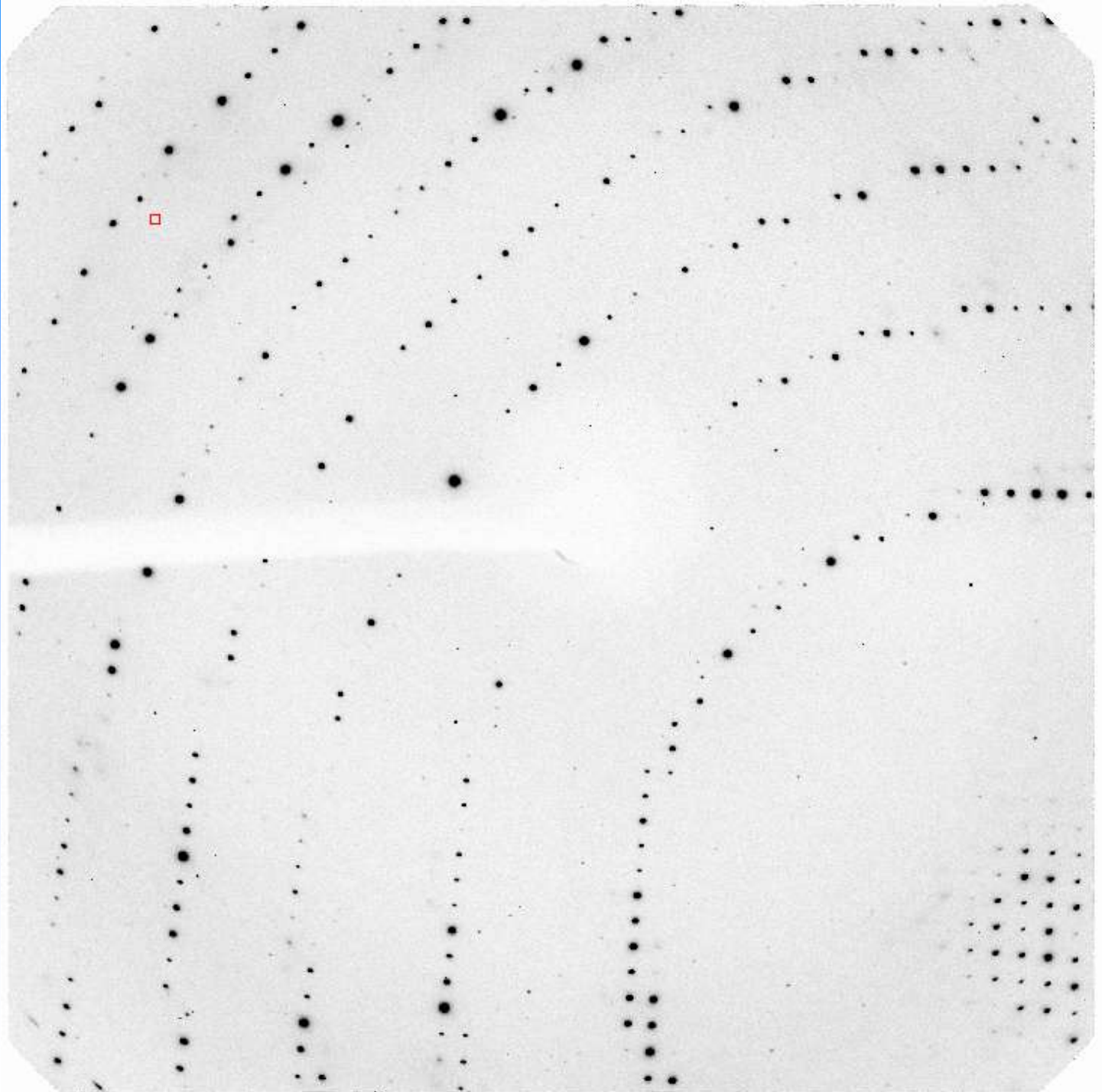
Let's look at a series of images from a CCD-based detector, each representing one degree of crystal rotation

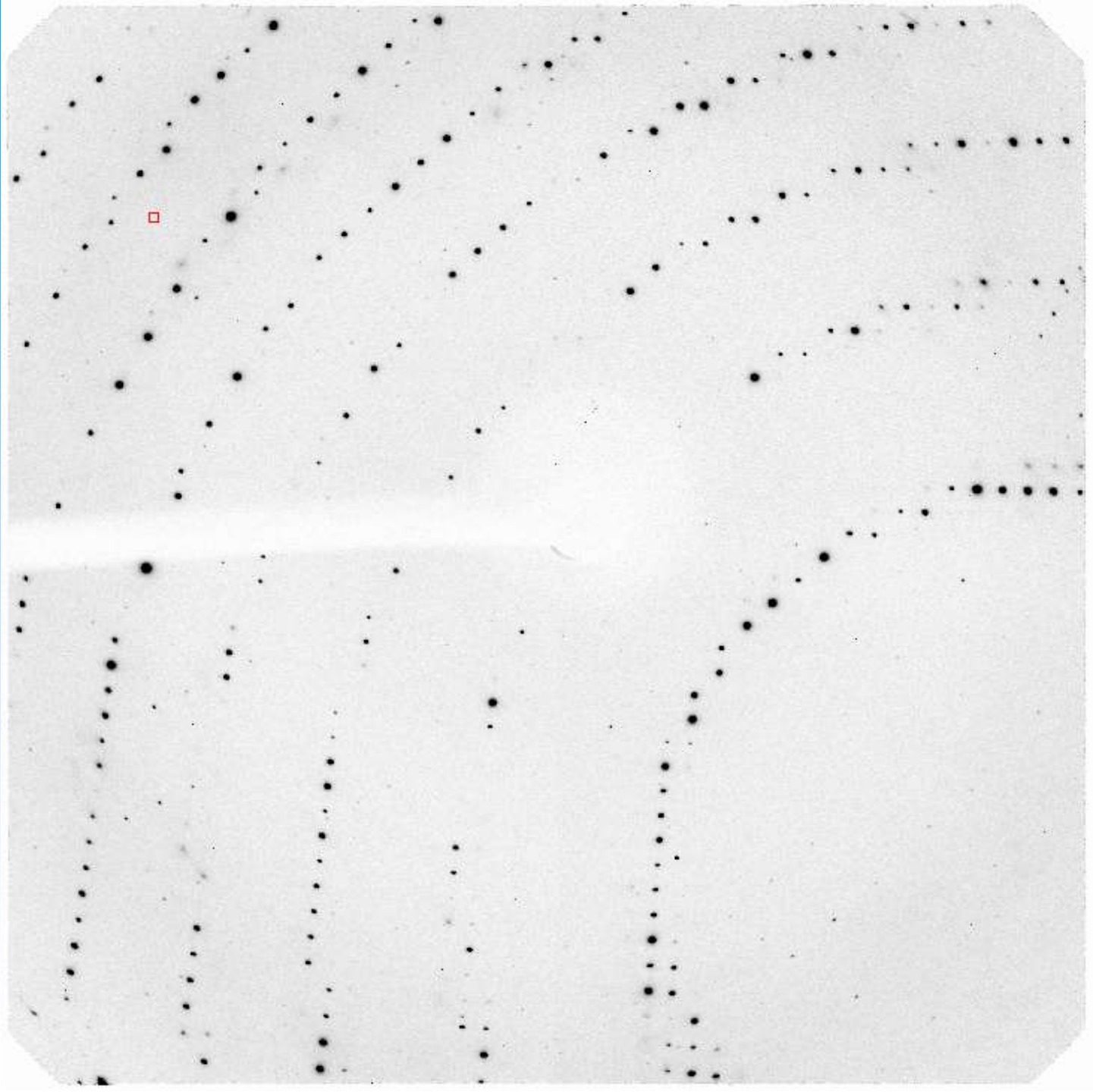


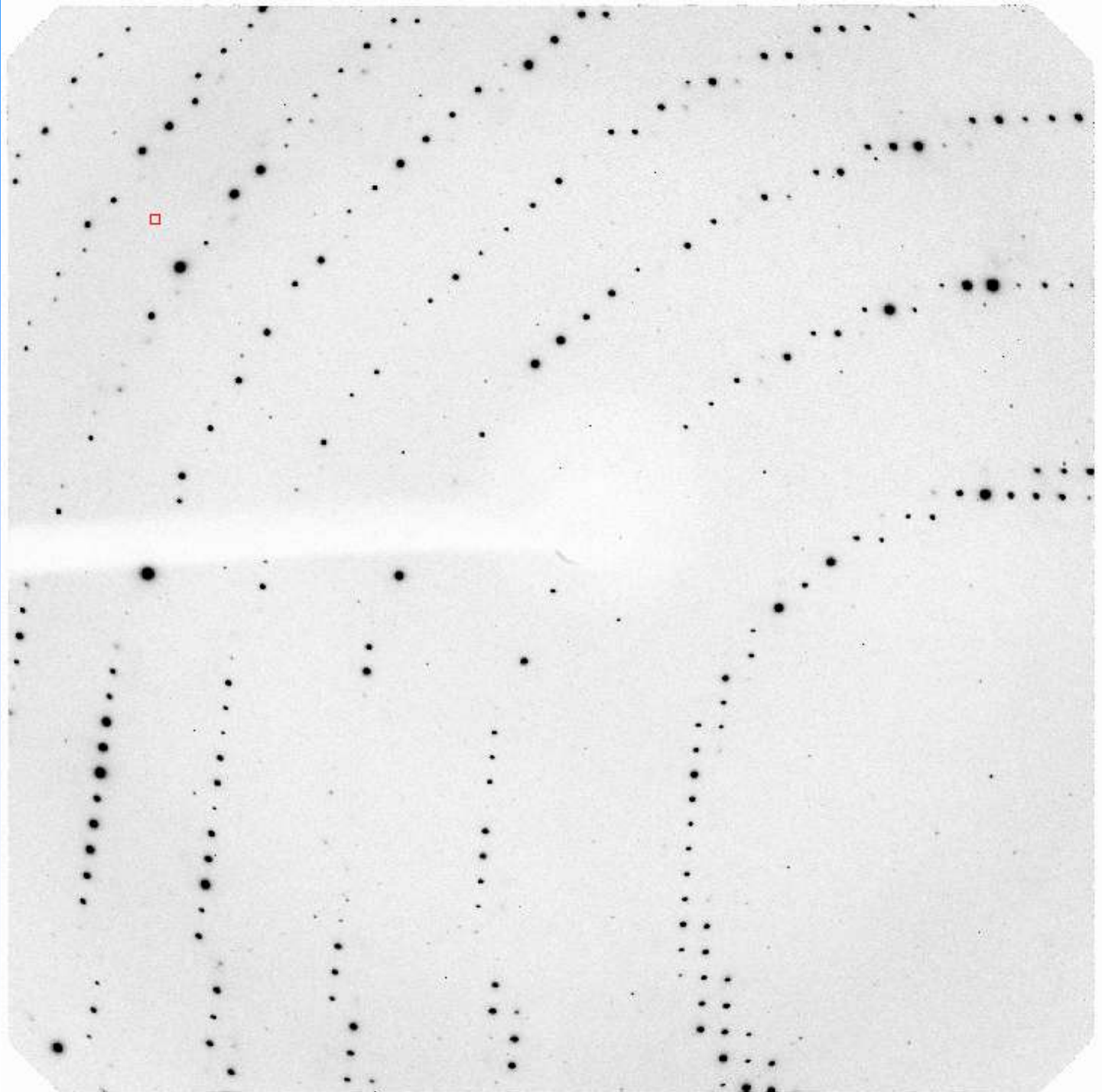


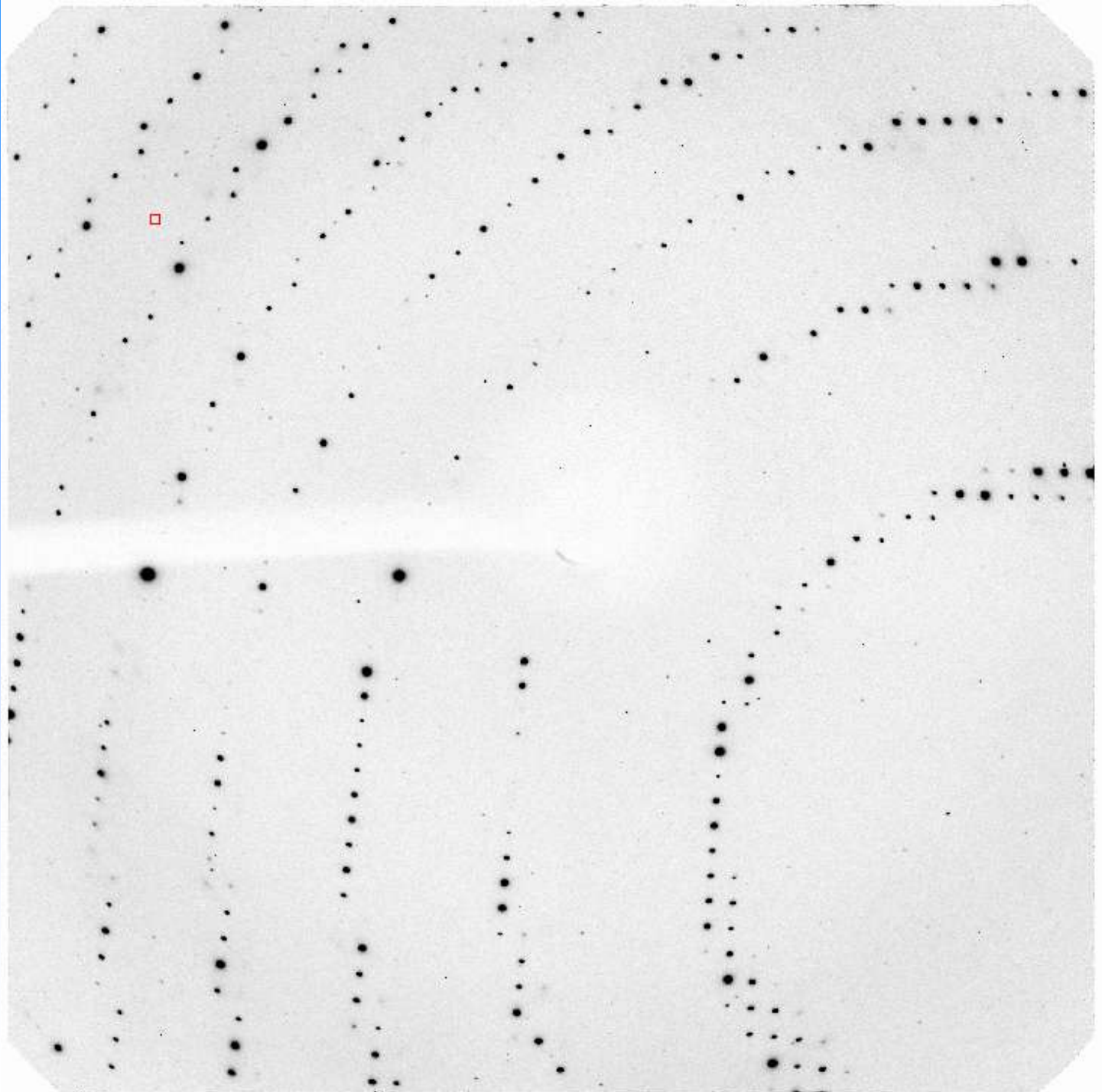






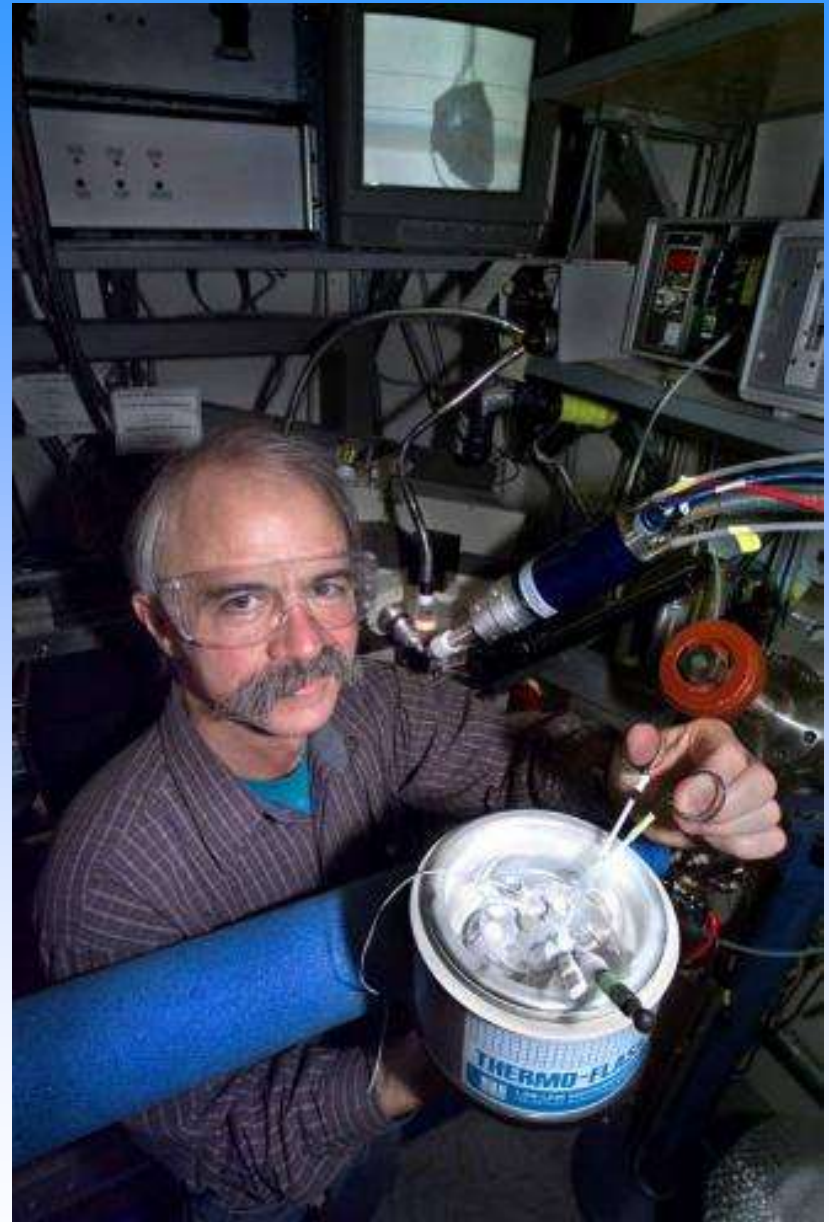






We use **cryogenics** to keep our specimens from being damaged by the x-rays.

These tools and containers are part of the apparatus we use with **liquid nitrogen** to produce cold temperatures.



The crystals are lifted from their mother liquor in these tiny loops of fairy hair.

The bases are cap-like and fit on a magnet on the x-ray diffractometer.

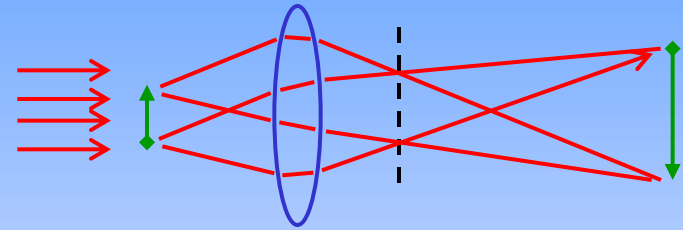


**There are four of these crystal-mounting
“robots” in operation at the NSLS.**



Q: How do we perform the second interference step in the functioning of the lens -- to **reconstruct the image** of the original object?

A: We will have to **calculate** it.



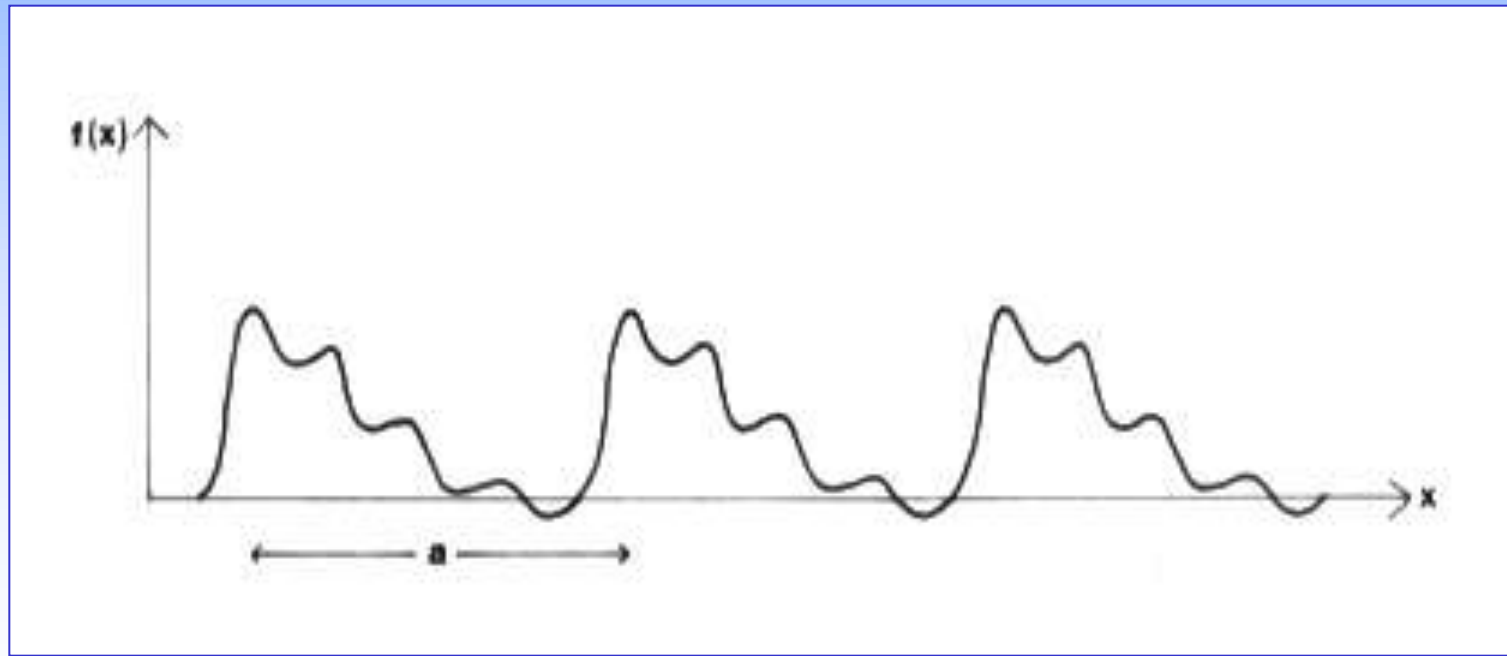
Q: How will we represent that object?

A: The **x-rays** are scattered from **electrons** in the atoms of the crystal.

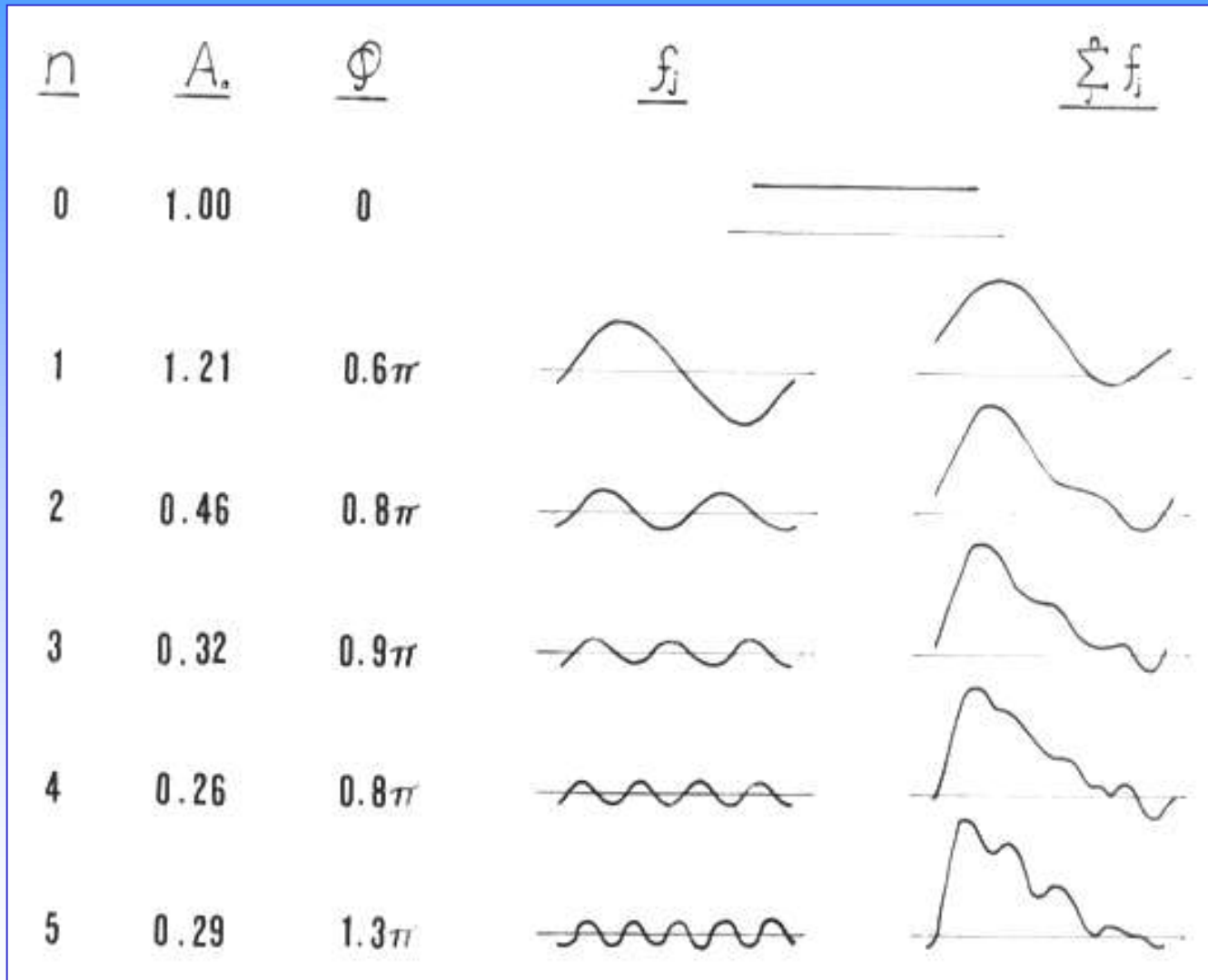
Therefore: for us, the “**image**” is going to be a representation of the **electron density**.

We use **Fourier synthesis** to calculate electron density. How does this work?

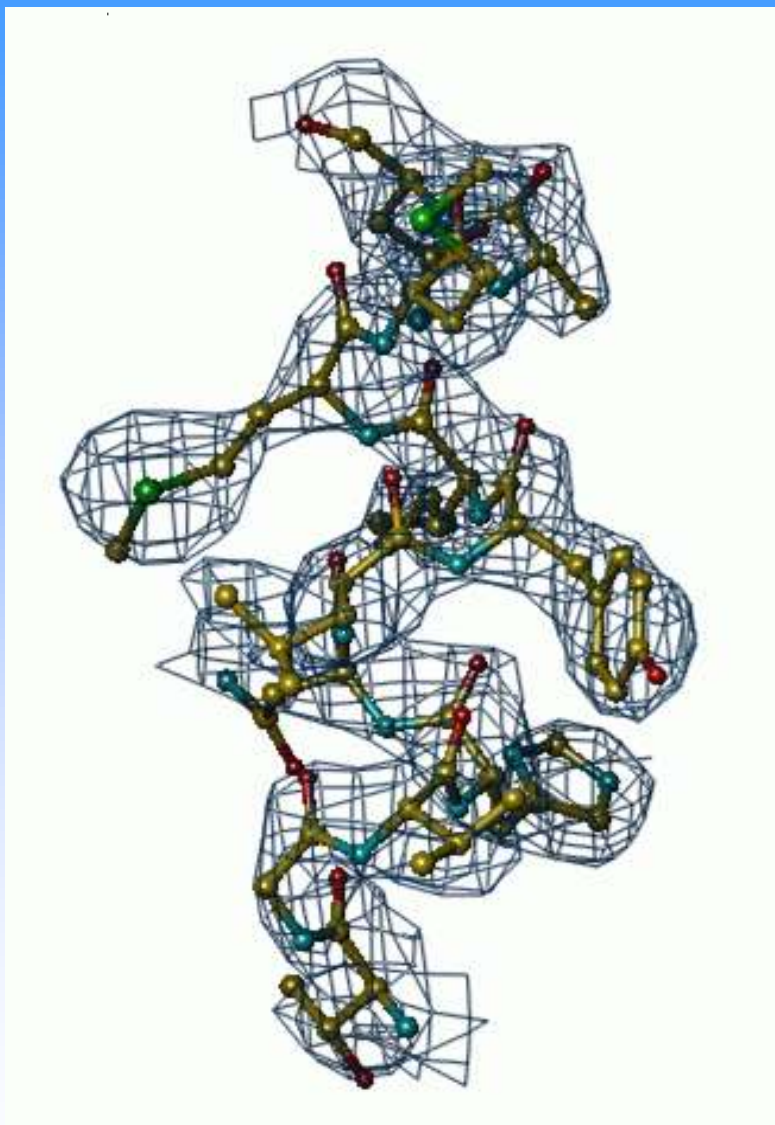
Can we produce a trial structure and see how waves can be summed to give this structure back?



In the **Fourier Synthesis**, just a few waves suffice to give a reasonable approximation to the original pattern.



S-adenosyl homocysteine hydrolase

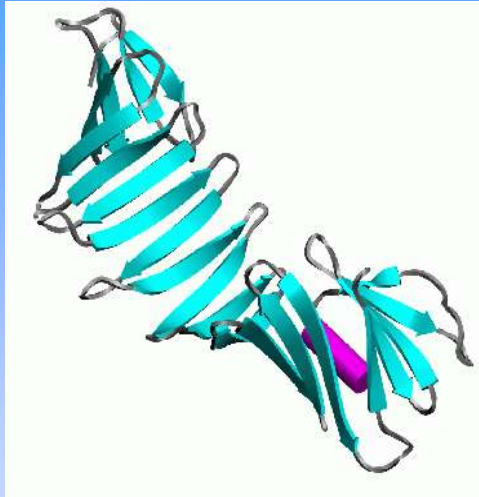


Typically, we represent regions of high electron density with “chickewire” cages like this.

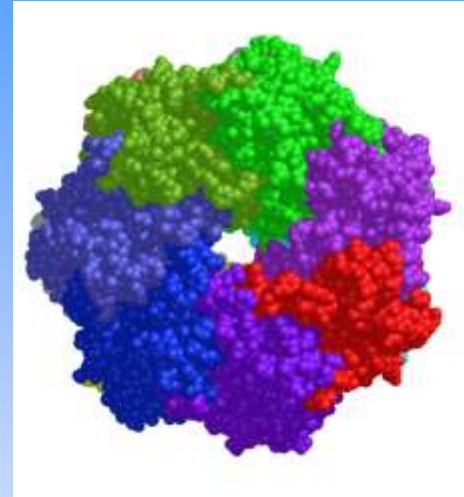
The molecular model can be built to fit inside.

BNL Research in Structure-Function

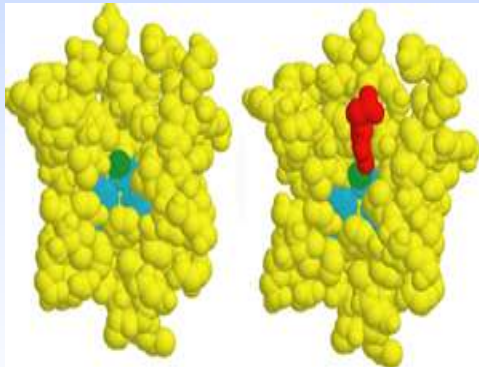
Lyme Disease
Vaccine
Protein



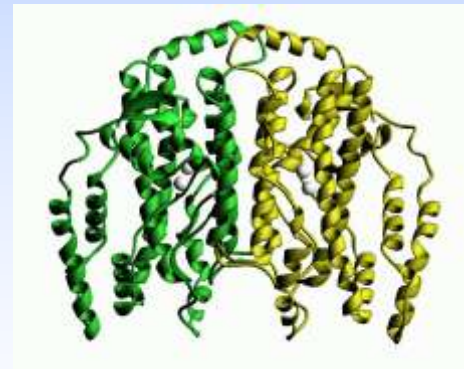
ClpP
Proteasome



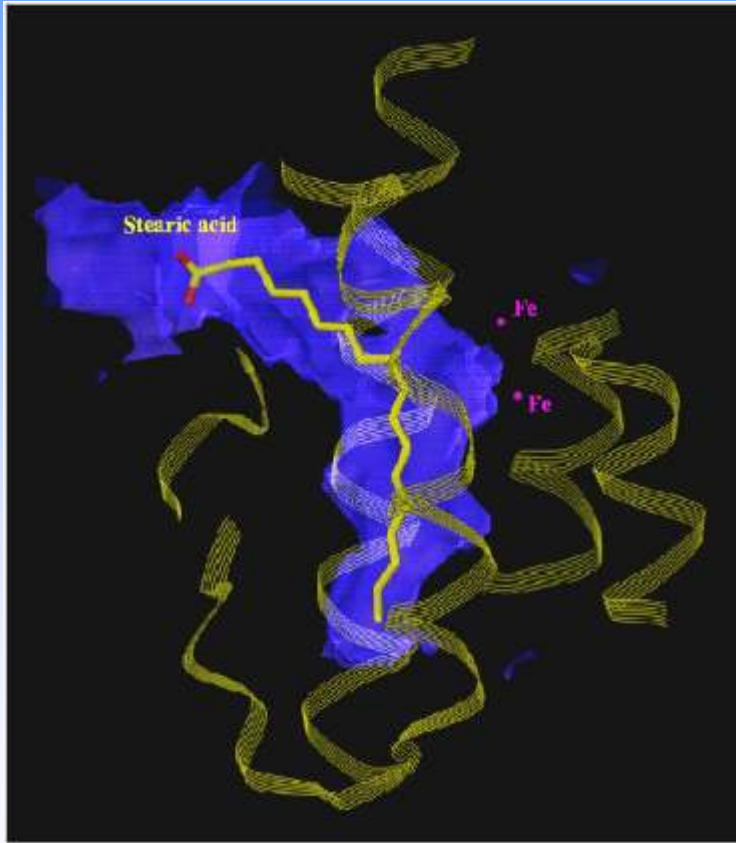
Human
Adenovirus
Protease
Drug
Interaction



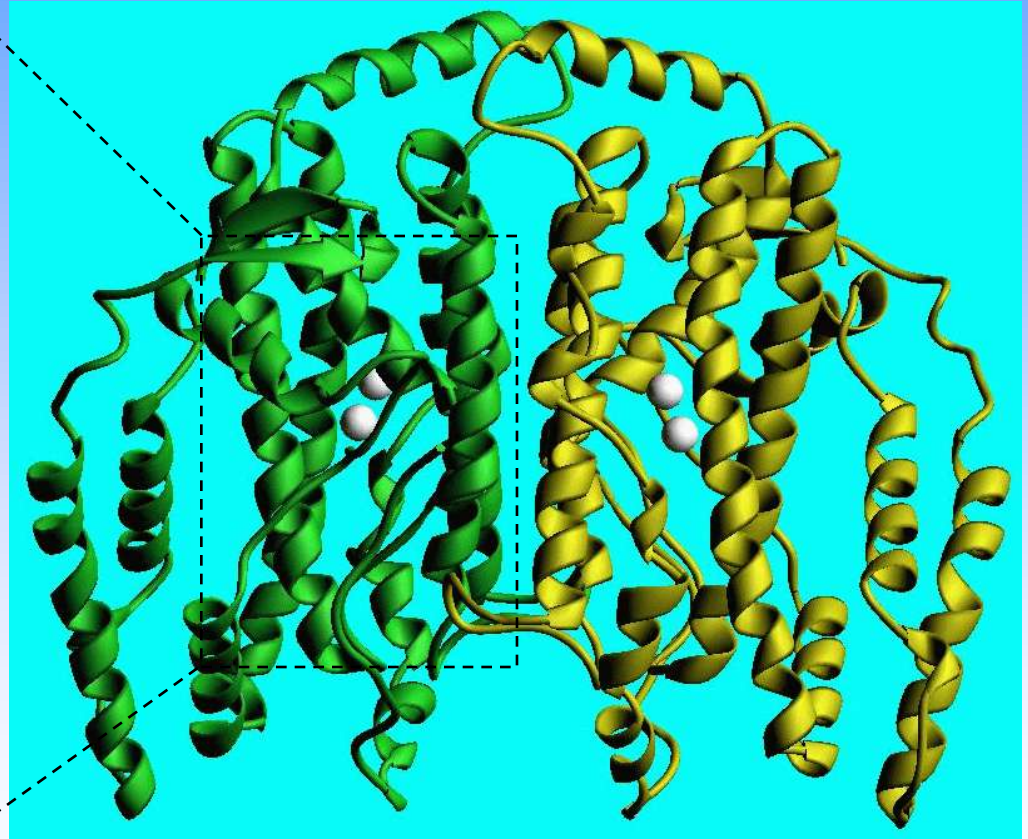
Desaturase



Plants use an enzyme called “**desaturase**” to convert saturated fats into unsaturated fats.



Active site cavity



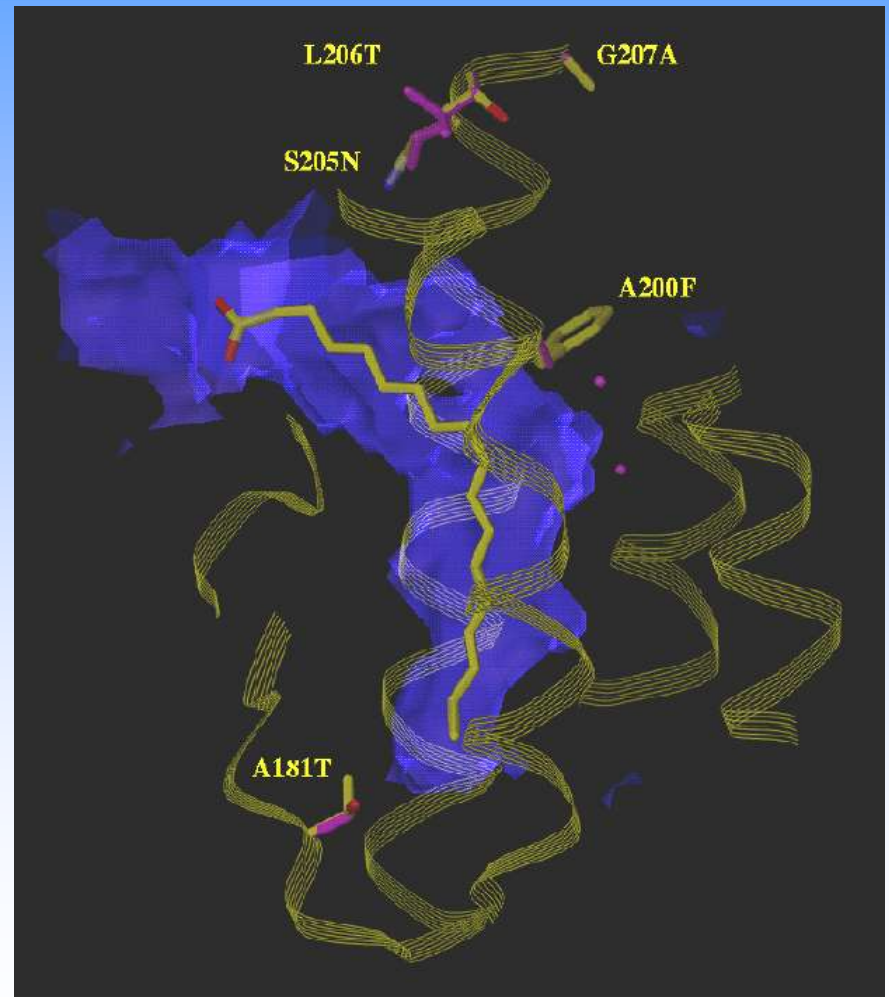
“Ribbon representation” of desaturase

Evolutionary studies help find **which amino acids** determine how a desaturase functions.

A crystal structure helps show how the chemistry of the molecule is determined by these residues.



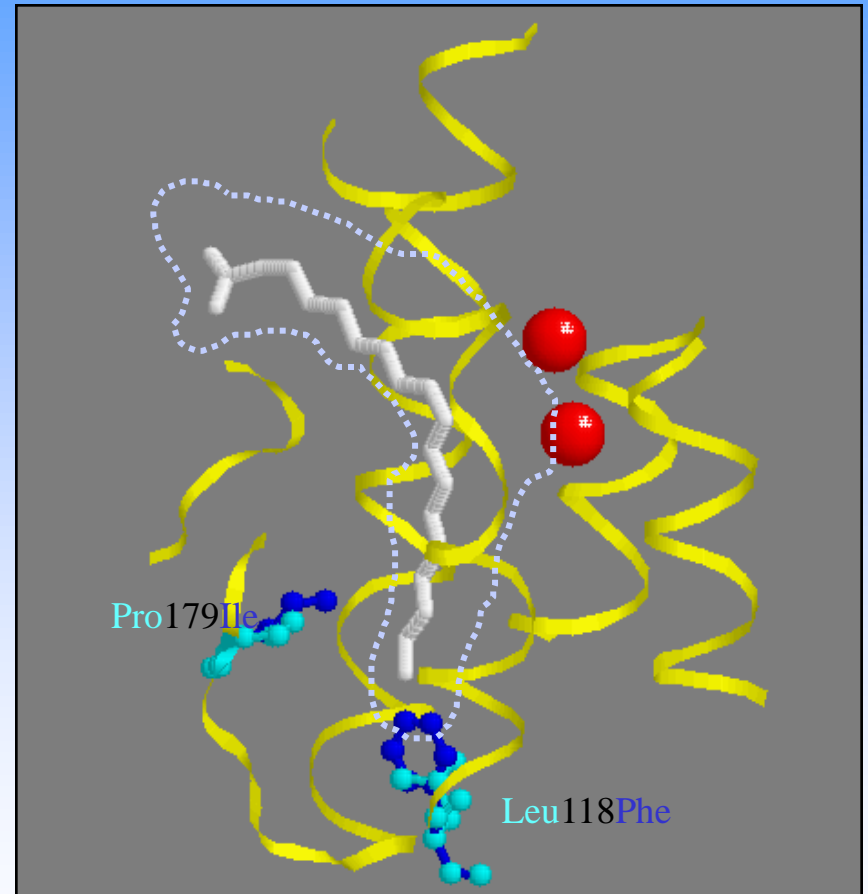
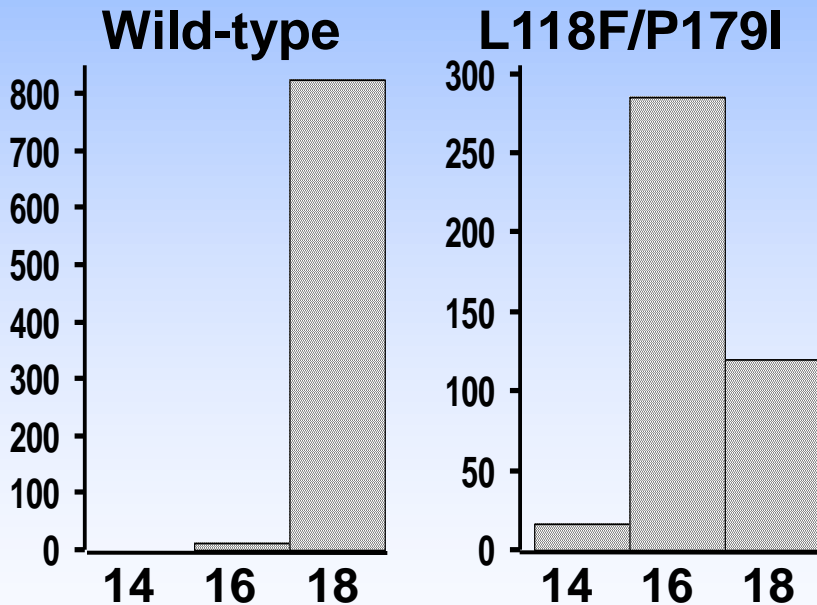
J. Shanklin



Brookhaven Biology

We can use genetic engineering to “redesign” a desaturase enzyme.

We can look at the enzyme’s structure and alter its shape, to change the way it functions.



By doing this, we could get more and **better quality** oils from crops like soybeans, canola, and sunflowers.

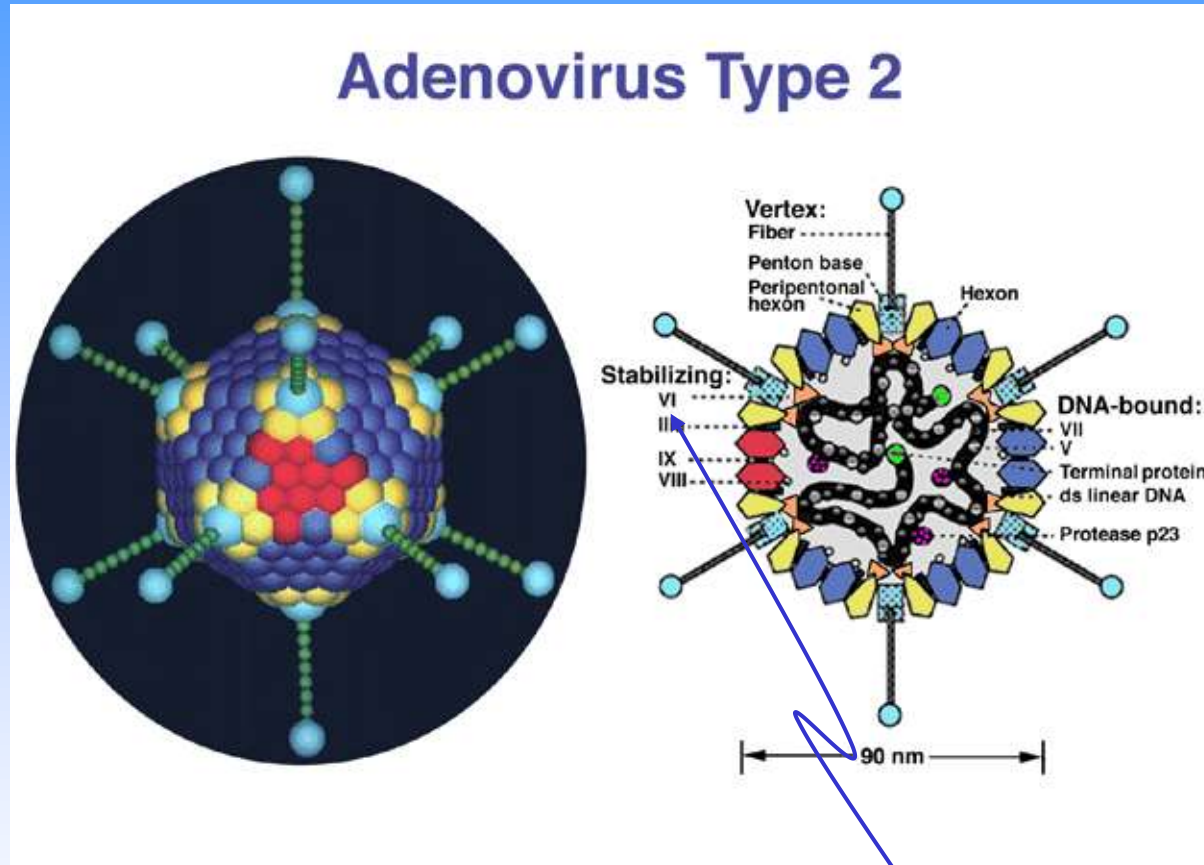


Improved plant oils
could mean more
nutrition from farm
crops...

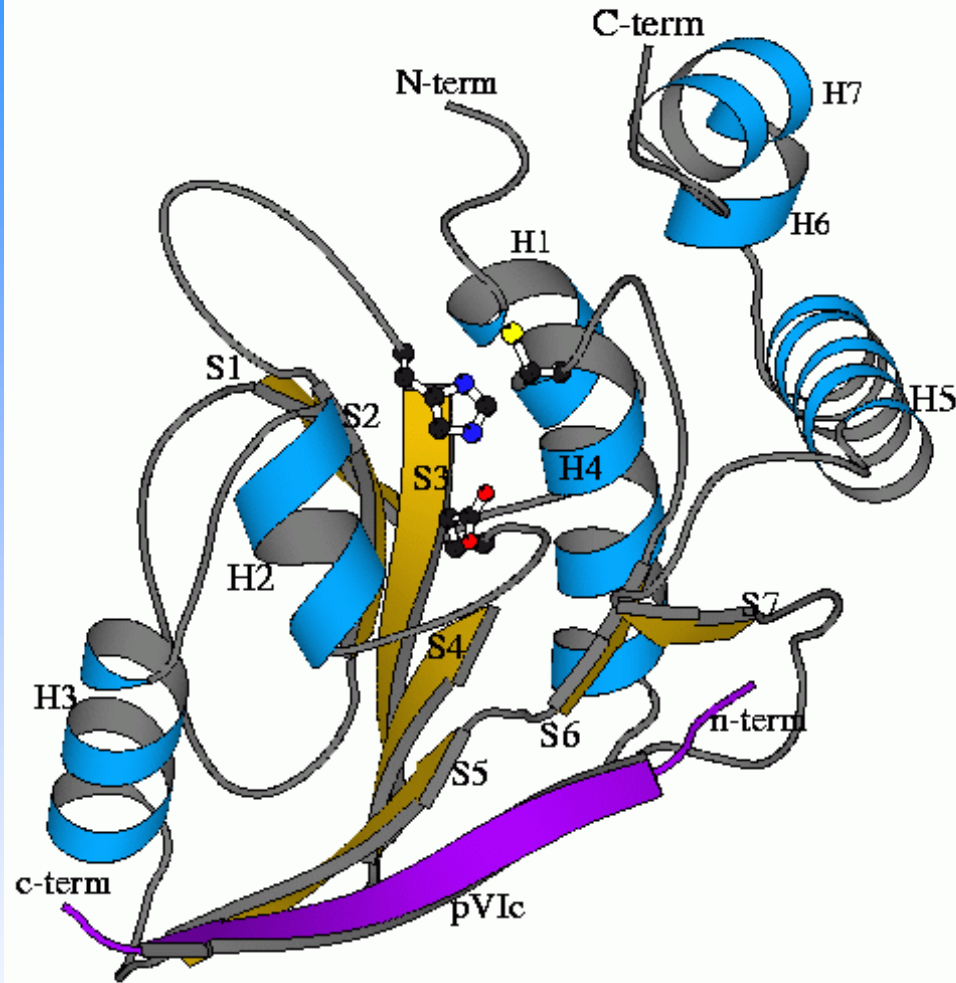
...or even new
renewable resources
that could take the
place of fossil fuels.

*Here are a couple more examples of
the science...*

Adenovirus causes cold-like symptoms in otherwise healthy people – worse diseases in weak ones.



The adenovirus protease primes pVI to form the infectious virus by cleaving it in a few places.



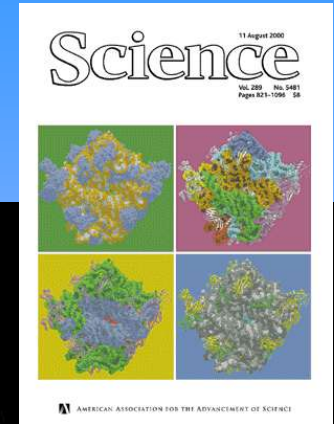
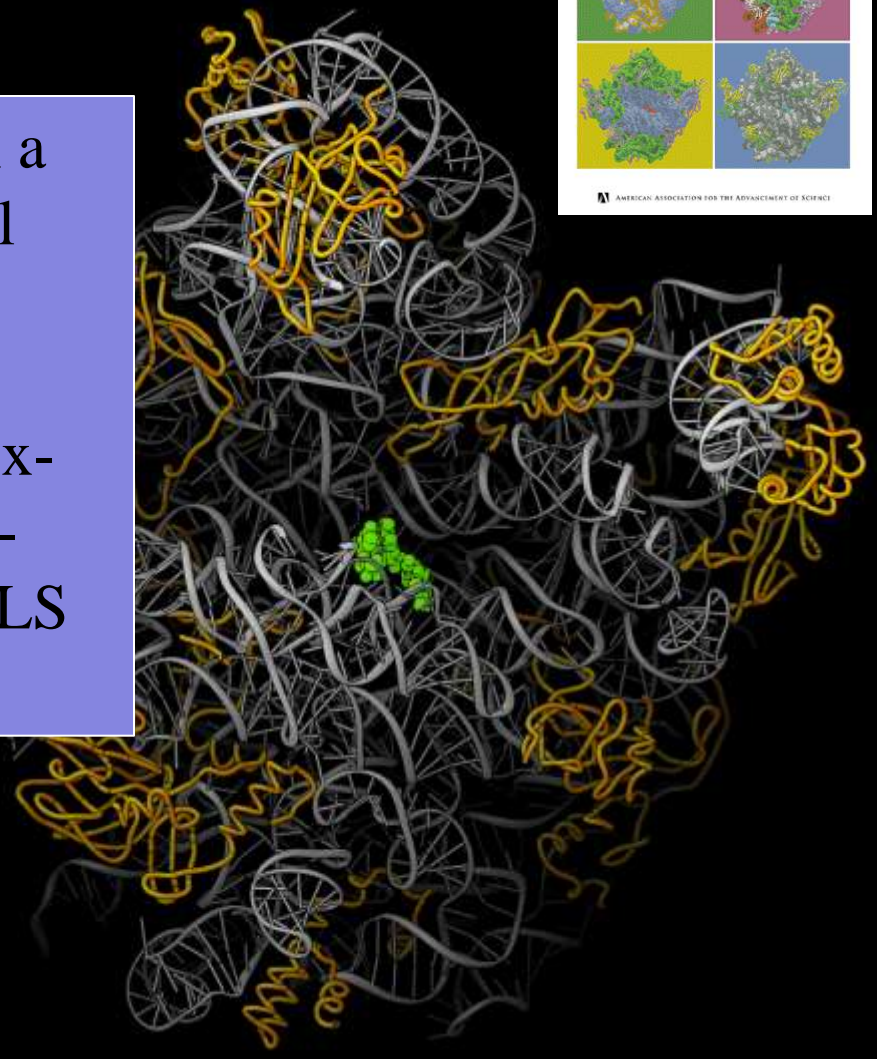
W. Mangel, W. McGrath, J. Ding, R. Sweet

And the protease itself is activated when the fragment pVIc binds to it to make the enzyme more rigid.

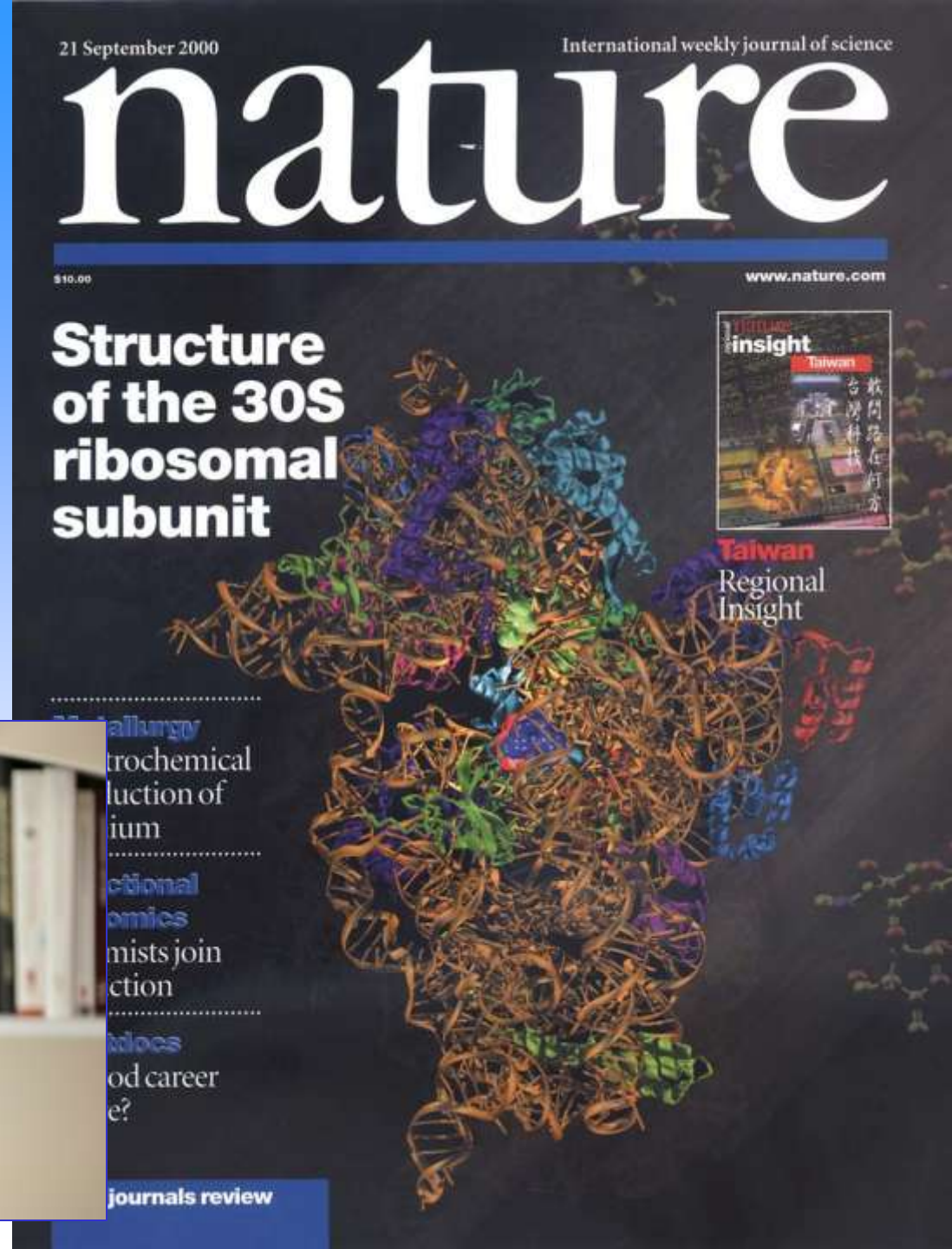
A landmark result for Brookhaven is this structure of the large subunit of the ribosome.

The increase in detail in structure thrilled us. This is precisely the cell's protein synthesis machinery. There are 100,000 atoms represented here!

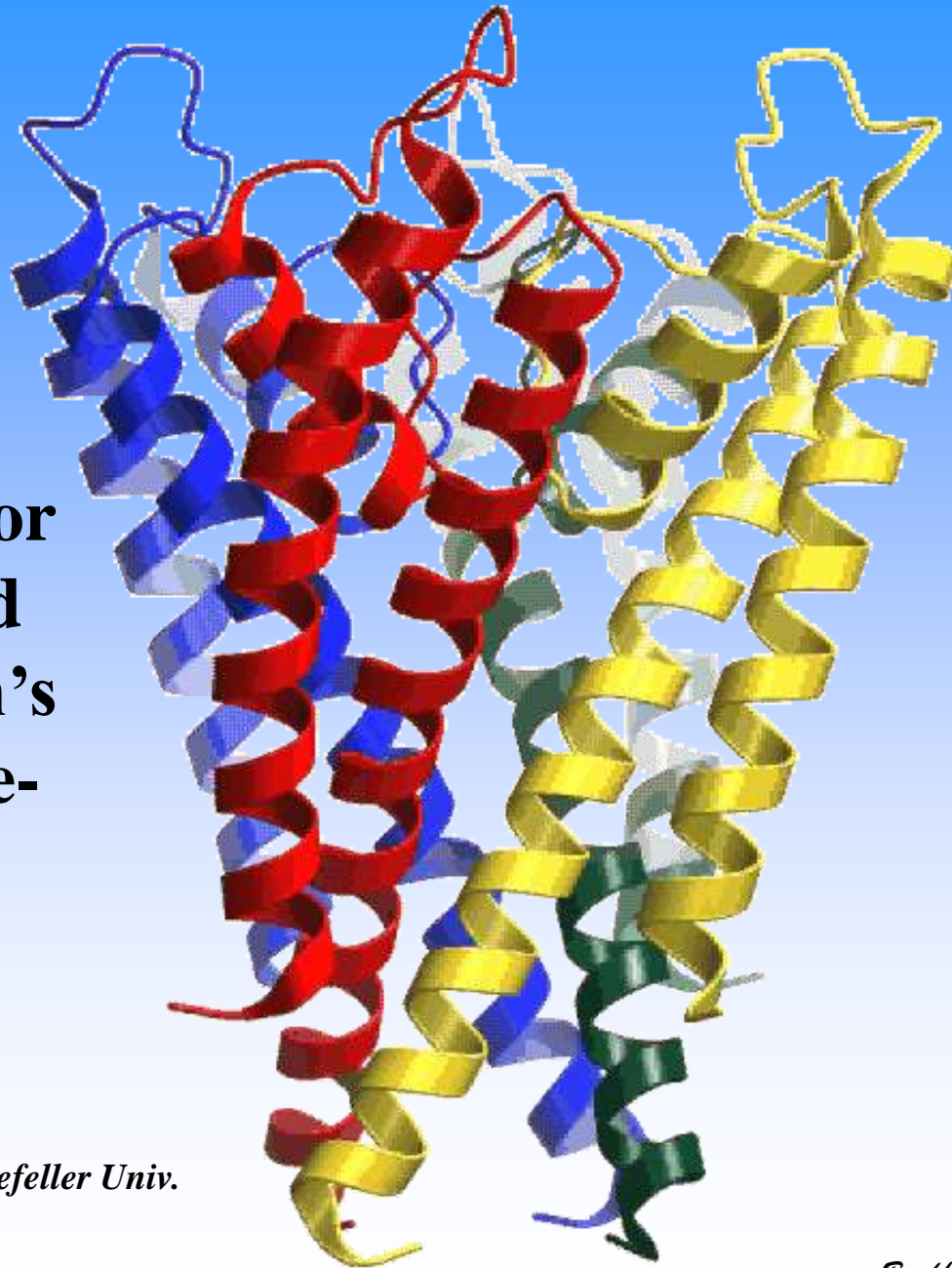
Tom Steitz of Yale won a share of the 2009 Nobel Prize in Chemistry for having determined this structure. Many of the x-ray diffraction measurements were done at NSLS beamline X25.



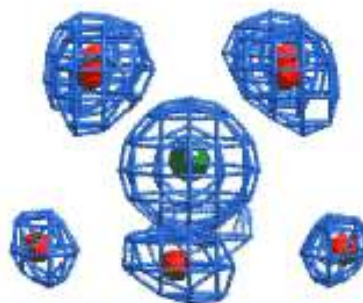
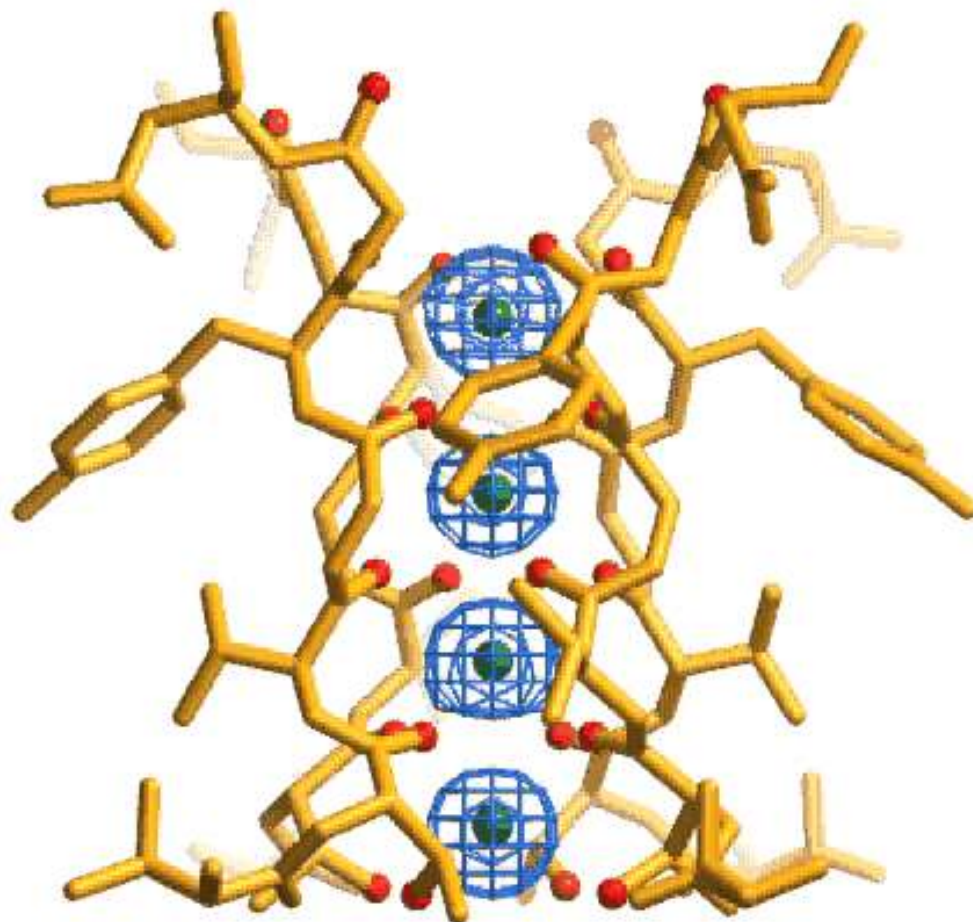
Venki Ramakrishnan of the Medical Research Council lab of Molecular Biology in Cambridge, UK shared this same prize. He was a Biology Dept. member from '83-'97. Did his early diffraction work at our beamlines X12-C and X25.



**Another
landmark for
us was Rod
MacKinnon's
Nobel-prize-
winning
work.**

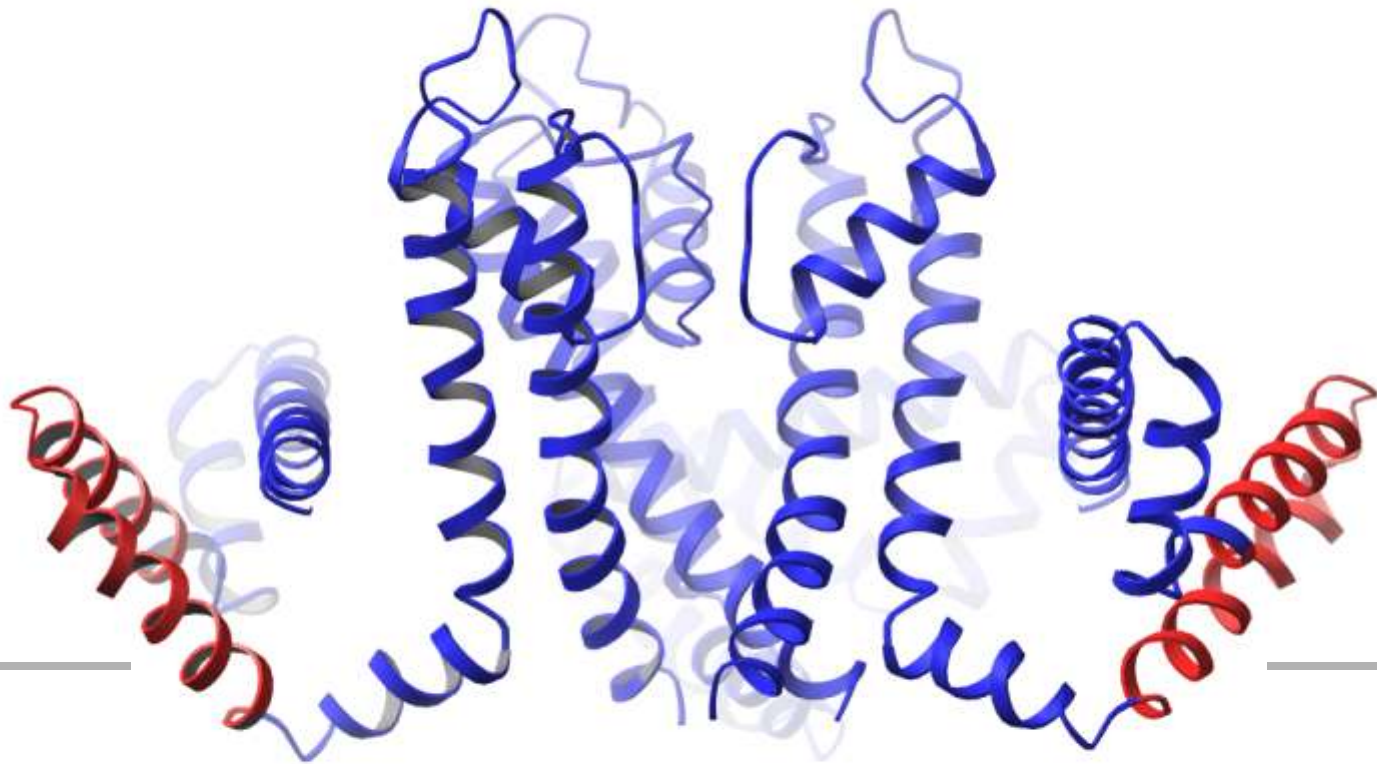


Rod MacKinnon, Rockefeller Univ.



Rod MacKinnon, Rockefeller Univ.

Closed

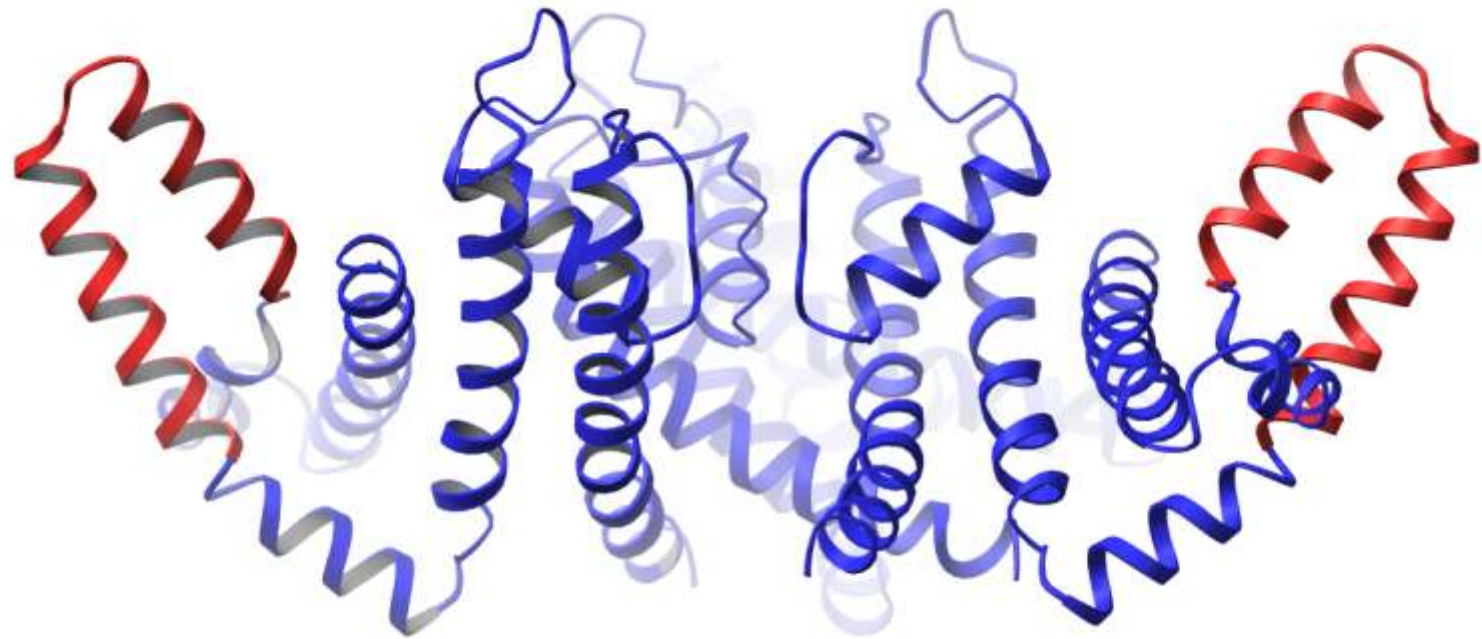


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Rod MacKinnon, Rockefeller Univ.

Opened

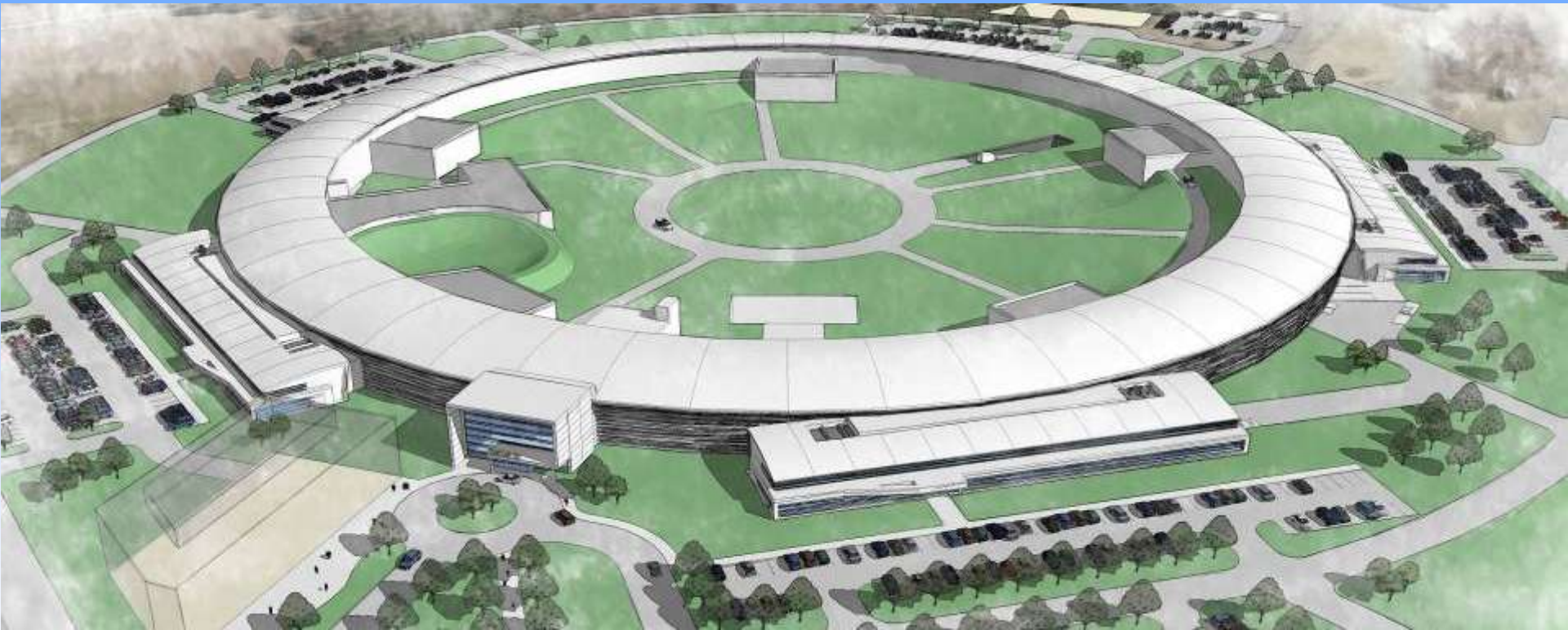


Rod MacKinnon, Rockefeller Univ.

Brookhaven Biology

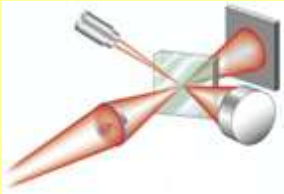
The Future Light Source for the Northeastern US

NSLS-II

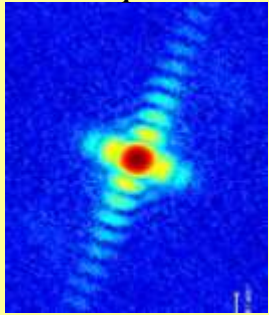


High Level Description of NSLS-II

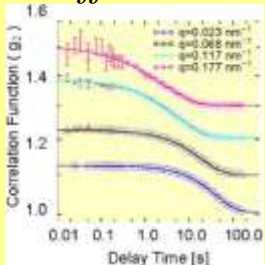
New



Nanoprobes



Diffraction



Coherent

Dynamics

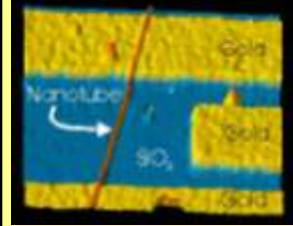
A highly optimized x-ray synchrotron delivering:

- *very high brightness and flux;*
- *exceptional beam stability; and*
- *a suite of advanced instruments, optics, and detectors that capitalize on these special capabilities.*

Together, these will enable:

- *~ 1 nm spatial resolution,*
- *~ 0.1 meV energy resolution, and*
- *single atom sensitivity.*

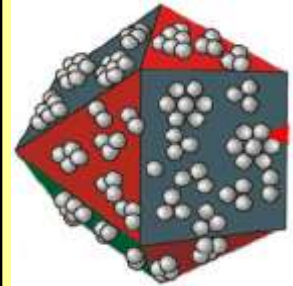
New Science



Nanoscience



Life Science

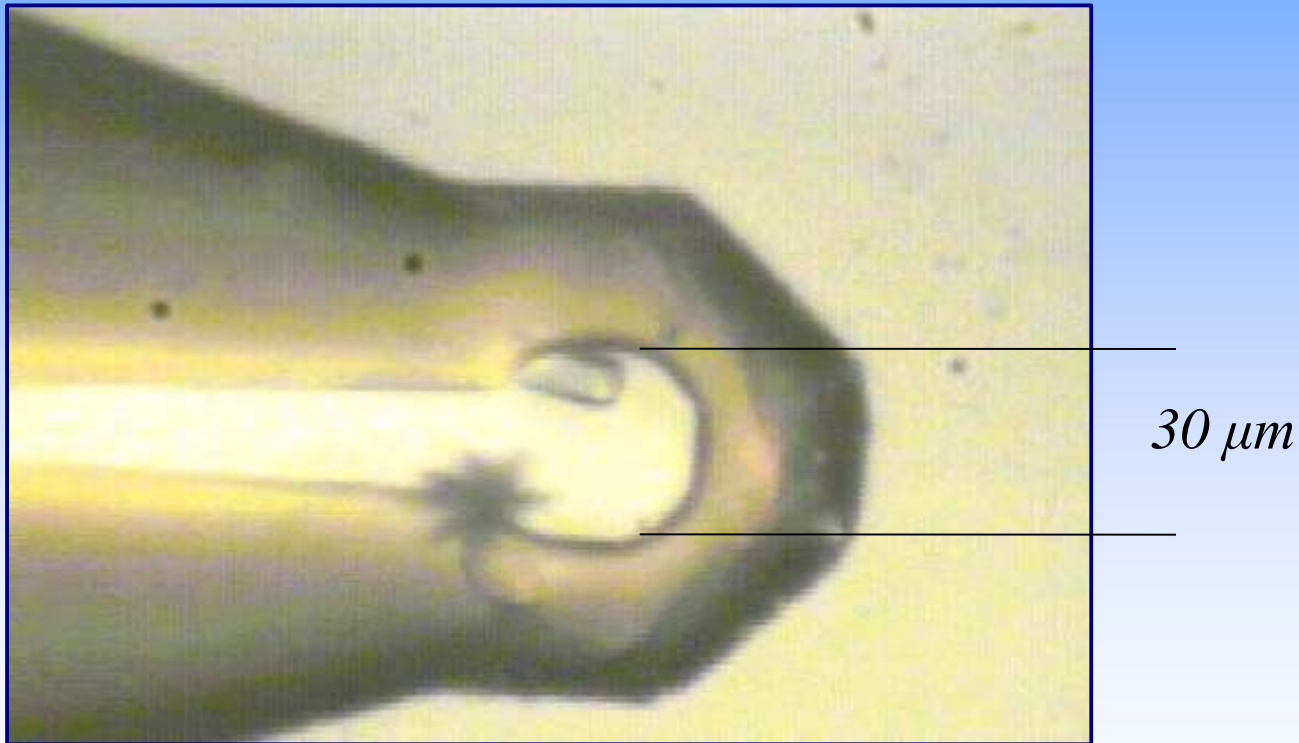


Nanocatalysis

Micro- beam diffraction

The modern 3rd generation sources easily produce beams in the 10 micrometer size range. A few can accomplish 5 micrometers, and we are planning one in the 1 micrometer range. How might these be useful?

Small beams can be used with **very** small crystals...



http://www.gmca.anl.gov/MiniBeam_for_WEB.pdf

Final Thoughts:

- We are in a golden age for biomedical research.
- Structural biology plays an important role by providing a tangible image of life processes.
- No single technique provides all the answers; we must collaborate.
- We also depend on collaboration among National funding agencies.

